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Technical Trip Report on Scientist Exchange Visit to Japan

September 29- October 28, 1981

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Introduction

During a four-week period from September 29 to October 28, I visited Japan to become familiar with the pine wilt disease situation, and with ongoing research on pine wood nematode, Bursaphelenchus xylophilus, and the pine sawyer, Monochamus alternatus, its principal vector. The general format of the visit was information exchange on the pine wilt disease in Japan and the United States, principally by discussions, presentation of seminars, and exchange of literature with Japanese scientists at the central headquarters of the Forestry and Forest Products Research Institute in Ibaraki, Japan, and at the Institute Branch Stations at Kumamoto (southern Japan), Kyoto (southcentral Japan),and at Morioka (northern Japan) (Appendix 1). Dr. Y. Mamiya, forest nematologist at the Forestry and Forest Products Research Institute, Ibaraki, Japan, was my principal contact before and during the exchange visit, and prepared my itinerary (Appendix 2).

In this report I have included general comments on forestry in Japan. Following these comments I have included information on locations visited, on discussions with scientists, on field trips, and on presentations of seminars.

General Comments - Japan Forestry

Japan is located east of Continental Asia between the Japan sea and the Pacific Ocean. It is comprised of the four main islands of Hokkaido, Honshu (the principal island), Shikoku, and Kyushu and of other minor islands. It stretches from northeast to southwest covering about 4,000 km, but the width of the main island is only about 300 km. The main islands are located in a zone 20 to 45° north latitude and have variable climate conditions from subtropical (southern extreme) to subfrigid (northern Hokkaido). The general climate, however, of most of Japan is warm-temperate and cool-temperate. About 75% of the land is occupied by mountains, hilly areas, and volcanoes. Since plains are scarce and used for farming, forested land is concentrated in the mountainous regions with steep terrain.

The forests of Japan are distributed in five groups: subtropical, warm temperate (as in Kyushu and Shikoku), cool temperate (north of the central part of Honshu), mixed conifers and broadleaves (in lowlands of Hokkaido's Oshima Peninsula), and subfrigid forests (mountainous areas of Hokkaido, and subalpine areas of Honshu and Shikoku).

The forest area of Japan occupies 68% (25 million ha) of the total land area, but the area per capita is only 0.24 ha. The domestic wood supply cannot satisfy demands and nearly 70% of wood used is imported. Efforts are being made to increase forestry resources in Japan by converting natural forests to planted forests that have high productivity.

In the 1940's forests of Japan were in a state of extreme devastation as the result of excessive cutting during and after the war. In 1950 the Japanese government enacted the "Tentative Reforestation Measures Law" which provided for regulation of forest cuttings and for reforestation of cut-over areas. As a result, replanting of cut over areas was almost complete by 1956, and efforts

since that time have been mainly in the so-called "expansion of reforestation" which is the conversion of natural broadleaf forests to artificial coniferous forests. The total area of artificial forests exceeded 9 million ha in 1975 and reached 10 million ha in 1980. Most of the artificial coniferous forests are not more than 30 years old because all were planted after the war.

About one third of the forest area in Japan is owned by the nation and under the control of the Forestry Agency; the remaining forest area is privately owned, and most of these forests (90%) are owned by individuals. The average size forest owned by individuals is only 2.6 ha. Nearly all individual forest owners are farmers who engage in forestry work when they are not busy farming.

Research Institutions and Scientists Visited

Central Headquarters
Forestry and Forest Products Research Institute
Ibaraki, Japan
Visited September 29-October 4, and October 26-28, 1981

Dr. Georges de Guiran--

During my visit to the Headquarters of the Forestry and Forest Products Research Institute, I was fortunate to have two discussions with Dr. de Guiran concerning the pine wood nematode distribution and incidence in France. Dr. de Guiran is nematologist, Station de Recherches sur les Nematodes, Institut National de la Recherche Agronomique BP 78, 06602 Antibes, Franche. He attended the 17th IUFRO World Congress at Kyoto (September 6-17, 1981) and remained in Japan to study specimens of Bursaphelenchus xylophilus and B. mucronatus in the laboratory of nematology before his return to France.

Dr. de Guiran is a competent nematologist who has done considerable research on plant-parasitic nematodes of agricultural crops. He was recently assigned to and is just beginning work on the pine wood nematode.

Dr. de Guiran indicates there are four species of Bursaphelenchus, namely B. lignicolus (synonym of B. xylophilus), B. leoni, B. naujaci, and B. pinasteri present in the "Landes", a forest of one million hectares planted with P. pinaster Ait. in southwest France(Baujard, 1980). B.lignicolus was originally found in 1981 in France and is now present in seven locations (Campet, Leon, Castets, Mimizan, Belhade, Pierroton, LePorge) in the "Landes" area (Appendix 3). Pine wilt disease is currently spreading at Campet and Belhade, and is distributed spordically at Leon, Mimizan, Pierroton, LePorge, and Castets.

Disease intensification has been slow in France. Since its initial discovery in 1971, Dr. de Guiran has indicated that it has spread only a short distance, and is not causing significant mortality in France. There appears to be several hypotheses for explaining this slow disease development. One hypothesis is that biological control agents regulate population of the vectors, but the insect or insects that vector the nematode in France are not known. Dr. de Guiran suspects that a cerambycid may be the vector. Another possible explanation for slow disease development is that the nematode may be B. mucronatus and not B. lignicolus since many female specimens have a micronate rather than a digitate tail. Dr. de Guiran plans to conduct mating tests between French and Japanese populations of B. xylophilus and B. mucronatus, and pathogenicity tests to resolve the taxonomic status of the French Busaphelenchus population. Although Baujard, another French nematologist, has synonymized B. mucronatus with B. lignicolus, other scientists, including Nickle et al (1981) and Dropkin (unpublished data) have confirmed that B. xylophilus and B. mucronatus will not mate in culture and thus are separate species.

Dr. de Guiran also mentioned that mortality is occurring in forests in southeastern France, and he plans to sample dead pine trees in affected areas to determine if B. xylophilus is present. Matsucoccus scales and man-caused fires are common problems in this area.

Dr. Yasuhara Mamiya--

Dr. Mamiya is Chief of the Nematology Lab, and he and Dr. Tamura are the only formally trained nematologists working for the Forest and Forest Project Research Institute. He spends about 30% of his time on administrative duties, and the remaining time is spent conducting research on the pine wood nematode.

Dr. Mamiya informed me that the Forestry and Forest Products Research Institute has recently started a third pine wilt disease project. The duration of the project will be 1981 through 1984. The director of the Institute is the leader of the project and Dr. Mamiya is serving as secretary. The specific objectives of the project are as follows:

(1) To reveal factors involved in disease development, especially physiological/chemical aspects.

(2) Determine how to produce toxins in or outside the host by using nematodes or other microorganisms isolated from or associated with nematodes.

(3) Determine the chemical characteristics of any toxins found.

(4) Determine the role of toxins in disease development, especially in the host.

(5) Study further the epidemiology of pine wilt disease. This objective will involve research to obtain more precise information on biology of Monochamus alternatus, and on the relationship of the vector and pine wood nematode. Some questions that scientists will seek to answer will include: Why do nematodes invade and leave adult M. alternatus? What triggers the change of nematodes from propagative state 3 to dispersal stage 3? What causes nematodes to change from dispersal stage 3 to dispersal stage 4? If molting from state 3 to 4 is chemically based, then perhaps chemicals can be used to change or disrupt this pattern.

The fifth objective is nearly half the entire project and will include considerable research effort on M. alternatus. Dr. Mamiya indicated that establishment of a pine wilt project means better financial support for conducting research, and a special budget for purchase of needed research equipment. Dr. Mamiya is scheduled to obtain a growth chamber in November 1981 from the special equipment budget. The equipment purchases are not financed out of operating funds.

There are 17 laboratories assigned to this project as follows:

Research Station	Division	Laboratory
Tsukuba	Forest Protection	1. Nematology
		2. Forest Pathology
		3. Forest Entomology Lab 1
		4. Forest Entomology Lab 2
		5. Pesticides
		6. Insect Pathology
	Silviculture	7. Silviculture
		8. Tree Physiology
	Forest Prod. Chemistry	9. Wood Chemistry
		10. Wood extractives
Kyushu	Forest Protection	11. Forest Pathology
		12. Forest Entomology
Kansai	Forest Protection	13. Forest Pathology
		14. Forest Entomology
Tohoku	Forest Protection	15. Forest Pathology
		16. Forest Entomology
Shikoku		17. Forest Protection

The smallest unit within the project is a laboratory, and all laboratories work together as a team. Objectives and a schedule of attainments have been developed for each laboratory. The budget for financing the project is divided equally among the 17 laboratories. Research results are reported annually during a general meeting, and scientists discuss work schedules and develop annual programs in view of research findings and project objectives.

The second pine wilt disease project was in existence from 1973 to 1976, and the first project from 1968 to 1971. The principal achievements of the first nematode project, as reported by Kobayashi (1981) were:

(1) Bursaphelenchus nematodes (described in 1972 as B. lignicolus) are a common inhabitant in wood of dead pine trees.

(2) Pine trees inoculated with the nematodes were killed within several weeks, and the killed trees had symptoms similar to those of naturally killed trees.

(3) B. lignicolus reproduces on cultures of some fungi.

(4) The Japanese pine sawyer, Monochamus alternatus, is the vector of the nematode, and one adult carries hundreds of thousands of B. lignicolus dauerlarvae in its tracheae.

(5) Nematodes are effectively transferred into the tissues through wounds made by the beetle during maturation feeding on pine shoots.

(6) Well-fed adults of M. alternatus lay the eggs onto inner bark of infected trees which have little oleoresin flow.

Dr. Mamiya is currently conducting research on the mechanism of disease development. In this study he is repeating some histopathological work to demonstrate the nature of host response in relation to feeding and reproduction of nematodes in wood, especially in the early stages of pathogenicity. His early work (Mamiya 1980) was conducted in 1976 with seedlings in the greenhouse, and his current work is with 7-year-old P. thunbergii. In this experiment he has removed bark from a small area of branches about 15 to 20 cm from the main stem, and inoculated these branches with 10,000 B. xylophilus in late July to early August 1981. He then pruned the inoculated branches flush to the main stem at 1, 3, 6, and 9 days after inoculation. He has not taken final field data, but observations made on

26 October 1981 show that some trees in all treatments were wilted or dead. The symptoms developed most rapidly in treatments where inoculated branches were pruned 3, 6, or 9 days after inoculation. Some trees in the 9-day treatment were already dead. These results are the same as obtained in his 1976 test and indicate that nematodes and some chemical, in response to nematode infection, moves into the main stem within 24 hours after inoculation and cause pines to wilt. He has collected samples from trees in each treatment for later histological study.

I viewed some colored slides and photographs of histological sections from his 1976 experiment,and his results indicate that some chemical factor is involved in disease development. In this work, branches of 3- year-old P. densiflora and P. thunbergii were inoculated with 5,000 B. xylophilus per seedling. He then collected inoculated and noninoculated seedlings 24 hours after inoculation and at intervals of 3 days for 15 days. He determined nematode populations from whole seedlings, and also prepared histological sections of wood samples from inoculated branches, from stems just below inoculated branches, from the base of the stem, and from the terminal shoot. His results (Mamiya 1980) show that death of ray and axial paranchyma cells is the first visible pathological response of pine to nematode infection during early stages of pathogenesis. Cell death occurred as early as 24 hours after inoculation and before reduction or cessation of oleoresin exudates which occurred on the sixth and ninth day after inoculation. Death of axial parenchyma cells was commonly observed following cessation of oleoresin exudation. The occurrence of cell death prior to nematode population increases and distribution indicates that pathological reactions of pine tissues to some chemicals might be involved in disease development.

Dr. Mamiya showed me a film on pine wilt disease that was made in 1976. This educational type film covers most aspects of the pine wilt disease situation in Japan, but some parts, especially on mechanism of disease development, are now out of date because of new research findings. Copies of the film are available for sale for 150,000 yen ($660.). The photography in this film is excellent, but there are no plans at present to revise it. I think this film would be a valuable training aid for forest pest specialists, forest pest management personnel, county agents, and extension personnel, and recommend its purchase if and when it is revised.

Dr. Mamiya has three review papers on pine wilt disease in preparation at the present time. The first is a chapter that includes a review of 300 Japanese papers on pine wilt disease. This paper will be part of a book on the history of forest pathology in Japan, and is scheduled for publication in March 1982. It will be written in Japanese. The second review paper is a chapter on pine wilt disease that will be part of a book on plant and insect nematology that is now being edited by Dr. William Nickle, USDA-SEA, Beltsville, Maryland. The third review will be a chapter on pine wilt disease that will be published in the Annual Review of Phytopathology, probably in 1983.

Dr. Hirotada Tamura--

Dr. Tamura was preparing to leave for Columbia, Missouri on October 8, 1981 to work with Dr. Victor Dropkin and colleagues of the Department of Plant Pathology, University of Missouri. Dr. Tamura will be at Missouri for a period of one year, apparently on a post doctoral grant. His research project may concern some aspect of toxins in pine wilt disease development.

Dr. Tamura has published reports on culture of Bursaphelenchus lignicolus under sterile liquid paraffin, and on callus cultures of pine and alfalfa. He has maintained cultures of nematodes for 1 year under this paraffin, and they have not lost their reproductivity or pathogenicity to pines (Tamura 1978). Dr. Tamura has also found that B. lignicolus is pathogenic on pines following 3 years storage on a fungus culture under sterile paraffin. He has some nematodes that have been in culture under paraffin for 5 years but has not yet conducted tests to determine if they are still viable and pathogenic to pines. He indicates that the key to long storage of nematodes is to keep them free of bacterial contamination.

Dr. Akiomi Yamane--

Dr. Yamane is Chief of Forest Insect Research Lab I. His research is primarily in the area of insect attractants. He has just completed a review article on the vector of pine wood nematode entitled "The Japanese Pine Sawyer, Monochamus alternatus Hope (Coleoptera:Cerambycidae): Bionomics and Control". The article will be published in 1982 in Review of Plant Protection Research by the Phytopathological Society of Japan, and the Japanese Society of Applied Entomology and Zoology.

Dr. Yamane and colleagues have investigated the chemical basis for the aggregation of B. xylophilus larvae around Monochamus alternatus pupal chambers. The migration of nematode larvae to pine sawyer pupae appears to be regulated by chemical and physical factors. In regard to chemical factors, Japanese scientists have shown that three unsaturated fatty acids (linoleic, palmitoleic, and oleic) and their glycerides are deposited on the pupal chamber walls from pine sawyer secretions and affect nematode larvae (Miyazaki et al 1977, Ikeda et al 1981). Dauerlarvae are also attracted by carbon dioxide released by

respiration of the pupae (Miyazaki et al 1978). The movement of dauerlarvae into tracheae of the pine sawyer is also related to carbon dioxide as an attractant (Ikeda et al 1981). In regard to physical factors, adults of the pine sawyer do not become infested with dauerlarvae when the water content of the wood is 20% or less because migration of the nematodes does not occur.

Certain chemicals have been shown to serve as stimulants in maturation feeding by the sawyers. Pine sawyer stimulants from current or 1-year-old twigs are B-sitosterol, campesterol, several sugars, sugar alcohols, and several glycosides(Ikeda et al 1981).

The pine sawyers are attracted to dying pine trees for feeding and oviposition by host alcohols and α - pinene. Dr. Yamane has used these chemicals in sticky traps to attract the sawyers (Ikeda et al 1980).

Entomologists of the Institute have conducted a number of studies on the development of the pine sawyers. These studies indicate that the sawyers require an accumulative temperature of 584 degree days heat accumulation above 11 C for their development. Dr. Ito at Kansai Branch Station in Kyoto is conducting work on development of pine sawyers on an artificial diet and may refine these temperature requirements. Dr. Yamane indicated that the pine sawyer is not present above Morioka in northern Honshu, and the limiting factor of its distribution is the requirement of 584 degree days heat accumulation above 11 C.

We also discussed predators and parasites of the pine sawyers. A number of papers have been published in a recent special issue devoted to natural enemies of the pine sawyer (Nobuchi 1980; Tamura 1980).

One quarantine report was of interest in respect to origin of B. lignicolus in Japan (Aihara et al 1980). This report includes a list of Bursaphelenchus species and other nematodes found in the wood of logs imported to Japan from several countries, including the United States. I did not see B. lignicolus listed in this report.

Mr. Tsukasa Kashiwa, Dr. Teiichi Fukami, and Mr. Kuniaki Matsuura--

On October 27, 1981, I visited with Mr. Tsukasa Kashiwa, Pesticide Section Chief; Dr. Teiichi Fukami, Chief of Pesticides Lab 1; and Mr. Kuniaki Matsuura, Pesticide Specialist, concerning chemotherapy work with systemic nematicides to control pine wood nematodes. Mr. Matsuura reported his research with nematicides and their effects on nematodes at the 17th IUFRO World Congress at Kyoto (Matsuura 1981). In research conducted at Chiyoda Experimental Forest in Ibaraki Prefecture, he has tested a number of nematicides during the last few years. Chemicals are applied into trunks of trees with injectors, or on the ground (granular formation) around the base of trees. Some 1 to 6 months after application, a nematode suspension is injected into trunks of treated trees. The effectiveness of chemicals is evaluated by determining the numbers of trees surviving per treatment the spring following chemical application and nematode inoculation.

For trunk injected chemicals, fensulfothion (Dasonit) has been effective in preventing tree death for three growing seasons when injected 6 months prior to nematode inoculation. When more than six ppm of this chemical is detected in branches of treated trees prior to nematode inoculation, no wilting occurs. Morantel tartrate (Banminth M) is also a promising systemic nematicide for protecting trees against pine wood nematode. This chemical is an antihelmenthic that is used for treatment of animals and thus is less toxic to wildlife than other chemicals under investigation. Mr. Matsuura has worked with this chemical for only a short time, and finds that it is effective against pine wood nematodes when more than 50 ppm is detected in branches and trunks of trees at time of inoculation with pine wood nematodes. Mr. Matsuura feels that the practical use of fensulfothion and morantel should be investigated further.

In determining dosage rates for application of systemic nematicides, Mr. Matsuura suggests that desired target concentrations in branches and stems be multiplied by a factor of 40 to estimate the concentration to be applied. For example, if a desired concentration of nematicides in branches and stems of a pine tree is 5 ppm, then a concentration of 200 ppm should be applied with trunk injectors. Mr. Matsuura finds that all nematicides move rapidly in an upward direction, and slowly in a downward direction in stems of trees.

For soil applied nematicides, methomyl and disulfoton are effective to some extent in reducing pine tree mortality. Mr. Matsuura has tested disulfolton in seven locations, (five in Honshu and two in Shikoku) and finds that it is effective against nematodes when concentrations reach 10 ppm in branches at time of nematode inoculation. The translocated portions of this chemical are the sulfoxide and sulfane forms.

Mr. Matsuura's current research on the mode of action of nematicides on nematodes reveals that the action is paralysis, and paralyzed nematodes apparently do not feed or reproduce. He suspended nematodes in solutions of morantel tartrate of concentrations of 0.2, 1, 5 and 25 ppm in containers and positioned the containers on trees so that nematodes had to pass through a small wad of cotton to enter wood tissues. Six trees were used in each treatment. The number of trees that died in treatments with 0.2, 1, 5 and 25 ppm morantel tartrate was 6, 3, 1, and 0 respectively. Nematodes were not affected in the 0.2 ppm morantel tartrate solution, but were paralyzed in the 25 ppm solution and did not pass through the wad of cotton into the wood of the pines and subsequently kill them.

Dr. Manabu Kusanoki--

Just prior to my return trip on October 28, 1981 I visited with Dr. Manabu Kusunoki who works in the forest pathology lab at the central headquarters. Dr. T. Kobayashi, Chief of this laboratory, was out of town at the time of my visit. Dr. Kusanoki graduated from the University of Tokyo in 1978 and has worked at the Institute since graduation. He is currently working on virus and mycoplasm diseases of trees, and indicates that the main virus diseases of trees in Japan are cucumber mosaic virus and tobacco mosaic virus. One of his research assignments is Paulownia witches broom, caused by a mycoplasm. He is currently trying to transmit the mycoplasm with insects. Two insects, Crytopeltis tenuis and Hishimonus sellatus (a leaf hopper) have reportedly transmitted the mycoplasm.

Seminar on Pine Wilt Disease in the United States

On October 1, 1981, I gave a 90 minute seminar to 25 scientists, principally entomologists and pathologists at the headquarters of the forestry institute. I discussed history of pine wilt disease in the United States, personnel and locations where research is being conducted, hosts, vectors, and showed many slides and discussed development of the disease in Illinois, Wisconsin, Minnesota, and in Missouri. The scientists were very interested in pine mortality caused by bark beetles in the United States prior to the discovery of the pine wood nematode, and the possibility that pine wood nematodes may have been associated with this mortality. In this regard I discussed Dr. C. Massey's findings of the 1950's and 1960's on nematode parasites and associates of bark beetles (Massey 1974

Field trips in Ibaraki Prefecture

On October 2, 1981 Dr. de Guiran, Dr. Mamiya, and I went on a 2-hour visit to Kukizaki Inashikigun to observe pine mortality caused by pine wood nematode. Damage to P. densiflora from pine wood nematode in this area, which is near the headquarters of the Forestry and Forest Products Research Institute (FFPRI), first occurred in 1977. Infected trees have not been removed from these stands because

they are located on private property. Approximately 30% of the pines observed in the stands were dying or dead, and Dr. Mamiya estimates that 50% of pines will be infected by 1982, and that all pines will be lost in 5 years if no measures are taken to control the pine sawyer and pine wood nematode. Loss of pines in this area is especially damaging because few or no other tree species occur in the understory.

On October 3, 1981 Dr. Mamiya and I took an all-day field trip in Ibaraki Prefecture to Mito and the Pacific Coast area. Pine mortality has been severe in this Prefecture and Dr. Mamiya discussed occurrence and progression of the disease. Pine wilt was initially found in Ibaraki Prefecture at Mito near the Pacific Coast in 1971 and progressed to the Tsukuba area by 1977. Pine wilt disease also spread into southern Ibaraki Prefecture from Chiba Prefecture. We observed extensive pine mortality that occurred within the last 3- to 4-years in Ibaraki Prefecture. In the area south of Lake Hinuma in Asahi-mura all pine trees have been killed. We observed many stands that were distinctly two storied; typically the overstory consisted of dead pine and the understory of Chaemacyparis obtusa or Cryptomeria japonica. Dr. Mamiya explained that Cryptomeria is more valuable than pines, and thus pines are not utilized. As a result, populations of nematodes and pine sawyers increased in infested areas, and many pines have subsequently been killed by the pine wood nematode. Pine mortality was especially heavy in 1978 (dry summer) when losses of 700,000 m^3 occurred in this Prefecture. This volume loss amounted to one third of all pine mortality (2,000,000 m^3) that occurred in 1978 in Japan. The total volume of pine wood in the 55,000 ha of pine forests in Ibaraki Prefecture is 7,500,000 m^3, and 1,900,000 m^3 has been lost to pine wood nematode from 1971 to 1980. Along

the Pacific seashore near Oarai we observed some pine mortality, and healthy P. thunbergii are commonly found. It appears that some environmental factor is preventing development of pine wilt disease near the seashore, and contrasts markedly with the 100% mortality that occurs in the interior of Ibaraki Prefecture.

Dr. Mamiya disclosed that 3.5 billion yen was spent in Ibaraki Prefecture in 1980 to control the pine sawyer. Trees were felled and their bark surfaces sprayed with insecticide (fenithrothion) to kill the pine sawyer before its emergence. This method of control was selected for use by the prefecture government. Dr. Mamiya explained that timing of insecticide application is critical for control of the vector. Infected trees must be felled and sprayed or removed before June following their infection in July-August of the previous year. It is not necessary to remove or spray infected or dead trees after the sawyer emerges in June because the sawyers do not return to oviposite in dead trees, and nematodes generally live only one year in logs after a tree dies. In Ibaraki Prefecture, insecticides have been applied in September or October in order to kill larvae of M. alternatus before they bore deep into pine wood. Log spraying at this time has not been very effective because agricultural crops are being harvested, and the supply of labor is very limited. Because of the lack of labor to spray logs in the fall, there is a need to find a more effective insecticide, method of application, or timing of application to kill beetle vectors before they emerge. In discussions with entomologists at Kansai Branch Station, I was informed that the sawyers may also be effectively controlled if logs are sprayed 2 to 4 weeks (early May) prior to their emergence.

During the morning of October 27, 1981, Dr. Mamiya and I visited the Chiyoda Experimental Forest of the Forestry and Forest Products Research Institute. This forest is located northeast of Tsukuba. Considerable pine mortality due to pine wood nematode has occurred in the forest and surrounding private land. In areas where older pines have been killed, younger pines are now being infected. Dr. Mamiya has not conducted any research on young pines at this forest because he feels that the pines could become infected naturally. The current research being conducted at this forest includes utilization of pine wood for production of charcoal, and use of systemic nematicides for control of pine wood nematode.

Observations of plantings of various tree species at the Chiyoda Experimental Forest indicate pines are the principal host of pine wood nematode. Larix leptolepis does not appear to be a host for B. xylophilus in Japan. I observed Larix trees in two plantings, one immediately adjacent to a P. thunbergii planting with current mortality, and the Larix were not infected.

Dr. Mamiya demonstrated how he collects samples from recently killed trees during this visit. His sampling equipment consists of an 18 mm drill mounted in a carpenters brace. He drills one or two holes 12 to 15 cm deep in the bole about 30 cm from the ground line. The wood fragments from the drilled holes are collected (about 10 g of material) and placed in a small plastic zip lock bag. These samples are then transported to the laboratory and processed for nematodes using the Baermann funnel technique.

Kyushu Branch Station
Forestry and Forest Products Research Institute
4-11-16 Kurokami, Kumamoto-shi
860 Japan
Visited October 7-10, 1981

I visited the island of Kyushu from October 5 to 10, 1981. On October 5, I observed pine mortality in Oita Prefecture, and on October 6 I traveled from Oita to Kumamoto. From October 7 to 10 I visited the Kyushu Branch Station where I had discussions with several scientists, presented a seminar on pine wilt disease in the United States, and visited the Kyushu Forest Tree Breeding Institute. Details of these events follow:

Kyushu, including Okinawa Islands, is located in the southwestern part of Japan (Appendix 1). The total land area is 4.4 million ha and the forest area is 2.76 million ha (62% of the total land area, including 1.46 million ha (53%) of man-made forest and 1.1 million ha (40%) of natural forests). The climate is humid warm. Annual mean temperature and precipitation are 17.6 C and 2300 mm respectively. Since this region is located in warm temperate and subtropical zones, trees grow rapidly and a wide variety of species are present. Shittake mushroom (Lentinus edodes (Berk.) Sing.) and bamboo are indigenous products of this region.

Pine wilt is the principal disease of pines on this island. Damage attributed to pine wood nematode was first recorded in a seaport at Nagasaki in 1905. In 1946 damage caused by pine wood nematode was observed in Oita Prefecture. At that time pine mortality did not develop extensively in Oita or surrounding areas because most dead and dying trees were removed and used as fuel. When petroleum fuels became available in the 1950's and 1960's, dying and dead pine trees were not utilized and as a result population of sawyers and nematodes increased. By 1961 the nematode had spread to the city of Oita,

and advanced to the Kunisaki penninsula by 1971. Since 1971, the disease has intensified in northern Kyushu, with extensive damage occurring in growing seasons characterized by high temperatures and low precipitation. Some 40,000 m^3 of the 4,500,000 m^3 of pine in Oita Prefecture have been damaged by pine wood nematode. Approximately 160,000 m^3 of the 18,000,000 m^3 of pine in Kyushu are similarly damaged.

I arrived at Oita airport during the morning of October 6 and spent the remainder of the day observing pine mortality in Kunisaki Peninsula and traveling to the city of Oita. I was accompanied by Dr. S. Yakota, Chief of Forest Protection Division, Kyushu Branch Station; Mr. T. Kiyohara, pathologist/nematologist, Kyushu Branch Station; Mr. Yukihiro Hagihara, Forest Protection Specialist with the Oita Prefecture Government; and Mr. Tomihiro Sakai, an employee of the Prefecture Government. We observed both maturation and oviposition feeding on P. densiflora. Adult M. alternatus emerge from May through August and transmit nematodes during maturation feeding in June and July under natural conditions. Maturation feeding usually occurs in current years shoots while oviposition feeding occurs on 3- to 4-year old stems. Susceptibility of pines to oviposition is dependent on environmental conditions; sawyers are not attracted to dying pines during periods of low temperature and rainfall. Symptoms of pine wilt disease appear in October in this area.

Most of the pine mortality we observed had occurred at elevations below 600 m. Apparently some mortality of P. densiflora caused by pine wood nematode has been found at 1100 m at Kirishima Mountains in southern Kyushu. The sawyer has a 2-year life cycle in high altitude areas where temperatures below 12 C prevail.

Mr. Hagihara explained the control program used by the Oita Prefecture Government to reduce losses from pine wilt disease. Since 1973 about 55,000 ha of pine stands have been aerially sprayed annually with two applications of fenitrothion (Sumithion^R). The first application of this insecticide to tree

crowns (late May) is timed to kill sawyers when they emerge and start maturation feeding on pine shoots. The second application(about mid-June) is applied at the time of peak emergence of the sawyers. Cost of this aerial spraying is about 45,000 yen per ha. At Imi in the northern part of Kunisaki Peninsula, we observed 1500 ha of pine forests that had been sprayed with fenitrothion twice annually for the last 4 years. The spraying program appeared effective, and no pine mortality was observed in the sprayed area. In some areas in Oita Prefecture, it is not feasible to apply insecticides aerially because of toxic effects to honeybees, silkworms, mulberry, and tobacco. In these areas, infected trees are felled and burned or sprayed with insecticides to kill the sawyers. Dying and dead trees in small, isolated,and scattered locations cannot be utilized because pine is worth approximately 8000 yen per m^3 while transportation costs to remove the pines are 20,000 yen per m^3. In individual areas where extensive mortality has occurred, it is economically feasible to utilize dead pine for pulpwood. The National Government does subsidize land owners for removal of dead pines from a fund appropriated for control of pine wilt disease. The Oita Prefecture Government also has quarantine regulations on movement of infected pine logs to help prevent spread of the disease.

On the trip between Oita and Kumamato on October 6, 1981 no pine wilt was observed from the highway because all pines had already been killed. Planted stands of Chaemcyparis obtusa and Cryptomera japonica are commonly observed on mountain slopes.

The Kyushu Branch Station of the Forestry and Forest Products Research Institute has ongoing research in silviculture and forest protection. There are 12 staff members of the forest protection division which is organized as follows:

Dr. Shun-ichi Yokota
Chief, Forest Protection Division

Forest Pathology Laboratory	Forest Entomology Laboratory	Forest Mycology Laboratory
Mr. Heiichi Hashimoto Chief of Lab	Mr. Taketami Chief of Lab	Dr. Ando Chief of Lab.
Mr. Tomoya Kiyohara Pathologist/Nematologist	Mr. Kuranaga Entomologist	Mr. Tsunoda Mycologist
Mr. Yuuji Kawabe Pathologist	Mr. Iwasaki Entomologist	
		Two Technicians
Mr. Yasuo Dozono Pathologist	Mr. Okochi Entomologist	

During the visit of the Kyushu Branch Station I had group discussions with Dr. S. Yokota, Mr. T. Kiyohara, and Mr. H. Hashimoto about research on pine wood nematode. I also had a discussion with Dr. N. Ooyama, Chief of Silvicultural Laboratory 1. I did not have sufficient time to discuss research on M. alternatus with entomologists. Aspects of some current research being conducted by scientists I visited follows:

Mr. Heiichi Hashimoto--

Mr. Hashimoto is Chief of the Forest Pathology Lab and currently spends about 50% of his time conducting research on systemic nematicides applied to the soil for control of B. xylophilus. In his 1980 test, he inoculated P. thunbergii with nematodes, and applied methomyl (lannate) when the first symptoms of pine wilt disease (yellowing of needle tips at the apex of the 1979 growth increment) appeared. The nematicide was applied at the rate of 100g of 45% active ingredient to 15 trees, followed by watering in for 10 days for uptake of the nematicide. Mr. Hashimoto indicates that time of application is critical; the nematicide must be applied when first symptoms appear on previous years growth. None of the inoculated trees that were treated with nematicide at this time has died during 1980.

In contrast, all trees inoculated with nematodes only died. Mr. Hashimoto explained that the nematicide did not kill all nematodes in inoculated trees. He found some live specimens in pockets of dead ray parenchyma of stems and roots of some trees. These trees died in 1981 when water stressed. Mr. Hashimoto has been able to detect methomyl in all tree parts by gas chromatograph analysis in 1980, and as early as 10 days after application from the terminal shoots of treated trees. He was unable to detect the nematicide from these treated trees in 1981. Thus the nematicide appears to be effective for 1 year. Mr. Hashimoto is collecting more research data from his test before making any conclusions on use of this nematicide. At present the experimental results must be regarded with caution.

Mr. Hashimoto has conducted some basic physiological tests on pines artificially inoculated with pine wood nematodes. He has found that symptoms appear three weeks after inoculation, and 10 days later (31 days after inoculation), transpiration has ceased. Nematodes increase rapidly at -20 bars xylem water potential, and the threshold value at tree death is -27 to -30 bars xylem water potential. Mr. Hashimoto has evaluated transpiration rates and numbers of xylem tracheids of inoculated and control seedlings grown in pots, and speculates that roots are affected more severely by pine wood nematode than stems. He added solutions of basic fuchsin to pots and after uptake of the solutions by seedlings examined cross sections of both stems and roots. He has a series of photographs that show all tissues of healthy seedlings stained at the root collar. Only cambial tissues of inoculated roots were stained; the central woody tissues of the roots were not stained and were apparently dead.

On the basis of these results he has concluded that water uptake by roots is very restricted in inoculated trees. This work will be published in the transactions of the October 1981 meeting of the Japanese Forestry Society (Kyushu Branch).

Mr. Tomoya Kiyohara--

Mr. Kiyohara is a competent forest pathologist who is spending all of his research time conducting studies on pine wood nematode. He and Tokushige (1971) did most of the inoculation work that led to the identification of the causal organism of pine wilt disease. While isolating fungi from wood of stems collected from dying pines, these investigators observed nematodes associated with fungal cultures. They inoculated three pine trees with nematodes in 1969-1970, and two died in 60 days. Further work revealed that the nematodes were a common inhabitant of wood of dead pines, and follow up inoculations with nematodes revealed that B. xylophilus was the causal organism of pine wilt disease. Since then many investigators have provided experimental evidence which shows a strong correlation between nematode inoculation and death of pine trees. It is fortunate that Mr. Kiyohara was not formally trained in nematology and did not have any preconceived ideas on how plant-parasitic nematodes feed on trees. A trained nematologist may have discounted nematodes as a causal organism of pine wilt disease without making any inoculations because nematodes were not known to survive and feed on cells in the xylem of trees at that time.

Mr. Kiyohara is currently conducting research on induced resistance to pine wood nematode in 6-year-old Pinus thunbergii. Most of his work consists of a pre-inoculation with an avirulent strain of B. xylophilus followed by a challenge inoculation with a highly virulent strain at specific time intervals after the pre-inoculation. In a 1980 test, he inoculated 10 trees 50 cm above the ground line with 30,000 avirulent pine wood nematodes and the pines did not die. When 10 trees were inoculated with 30,000 virulent nematodes, or with 30,000

avirulent and virulent nematodes simultaneously, all trees died. However, when the challenge inoculation was made 5 or 21 days after the pre-inoculation, 60 and 90 percent of the trees survived respectively. Results thus suggest that when the interval between pre-and challenge inoculations increase, more trees survive, but the mechanism involved in the increased resistance to virulent nematodes is not known.

Mr. Kiyohara conducted five additional tests in 1981 to further investigate induced resistance to pine wood nematode. In one experiment to determine if resistance could be induced systemically, he made pre-inoculations (30,000 nematodes) on branches of 3- 5- and 15-year-old P. thunbergii, and challenge inoculations 30 days later either above or below the pre-inoculation. Results indicated that resistance was induced systemically regardless of location of the challenge inoculation. Results from other treatments (pre-inoculation only, challenge inoculation only, and pre-and challenge inoculation simultaneously) were the same as obtained in the 1980 test. Trees inoculated with 30,000 avirulent nematodes did not show foliage symptoms or die, but did exhibit reduced resin flow. Mr. Kiyohara examined nematode populations from some trees that received both pre-and challenge inoculations, and found very low populations. However, he could not identify recovered specimens as either avirulent or virulent nematodes.

In a second 1981 test, Mr. Kiyohara used B. mucronatus and four unidentified Bursaphelenchus species to see if resistance could be induced systemically with these species. Results showed that resistance to pine wood nematode is not induced by any of these species. In a third 1981 test, Mr. Kiyohara used 10, 30, and 360 days as intervals between pre-and challenge inoculation. Results for treatments with 10 and 30 days between inoculations

confirmed that resistance to pine wood nematode could be induced. The 30-day interval (95% survival) was more effective than the 10-day interval (75% survival) for improving tree survival. Many trees in the 360 interval treatment died (33% survival), and Mr. Kiyohara speculated that the original 30,000 avirulent nematodes died and remaining virulent nematodes killed the trees. Mr. Kihohara has investigated further the interval between pre-and challenge inoculations on survival of 5-year-old P. thunbergii in a fourth test. In this test, intervals between pre-and challenge inoculations were 30, 60, and 90 days. Twenty trees were used in each treatment, and results suggest that as the interval between pre-and challenge inoculations is increased, the percent survival of trees increases (90 day = 75% survival; 60 day = 60%; 30 days = 50%; control inoculation with virulent nematodes = 15% survival). In the fifth 1981 test, Mr. Kiyohara investigated the effects of inoculum density on induction of resistance by pine wood nematode. Twenty to 22 trees were used in each treatment. Results show that when pre-inoculations were made with 30,000 avirulent nematodes, and challenge inoculations are made 30 days later with 100, 1,000, or 10,000 virulent nematodes, survival is inversely proportional to population level of challenge inoculation (100 inoculum level = 86% survival; 1,000 nematodes = 77% survival; and 10,000 nematodes = 50% survival). When pre-inoculations were omitted, and challenge inoculations made with 100, 1,000, or 10,000 virulent nematodes, tree survival was 28, 28 and 15% respectively. Results of Mr. Kiyohara's 1980 and 1981 tests have revealed that resistance to pine wood nematode can be induced systemically, but the mechanism involved is not known. Mr. Kiyohara will be conducting more research to clarify the mechanism involved.

Dr. Namio Ooyama--

I also had a discussion with Dr. Namio Ooyama, Chief of the first silviculture laboratory, on breeding pines for resistance to pine wood nematode. The discussion was based primarily on the publication "Studies on the systematization of integrated control of pine wood nematode" by M. Nishizawa and N. Ooyama, 1981. The manuscript was compiled by the Institute of Forestry, Department of Agriculture,Kyushu University. Drs. Nishizawa and Ooyama summarized over 70 papers on resistance studies to pine wood nematode in Japan. Tree species designated as strongly resistant to pine wood nematode include Pinus taeda, P. pungens, P. virginiana, P. strobus, P. banksiana, P. clausa and P. bungeana. Tree speices that are moderately resistant include P. elliottii, P. rigida, P. rigitaeda, P. massoniana, P. tabulaeformis, and P. echinata. Pinus densiflora is susceptible, and P. thunbergii, P. luchuensis, P. pinaster, and P. pinea are designated as very susceptible to pine wood nematode. Dr. Ooyama indicated that the resistance of P. taeda is variable according to provenance location of seeds. Trees from seed obtained from the coastal range of P. taeda in the United States are less resistant to pine wood nematode in Japan than those from interior United States.

In resistance tests now being conducted in Japan grafted seedlings are routinely used because such seedlings provide greater uniformity of genetic material. In earlier resistance tests 3-year-old seedlings were used. The standard procedure for all resistance screening tests with pine wood nematode is to inoculate grafted seedlings 30 cm above the graft on the scion. Nishizawa and Ooyama (1981) have cited research conducted in 1975 on susceptibility of the graft union to pine wood nematode that shows scions (P. taeda and P. elliottii) are much less susceptible to inoculation with 30,000 nematodes than root stocks (P. thunbergii). The research also shows that scions and stocks of P. densiflora and P. thunbergii were very

susceptible to nematode inoculation. The nematode populations of whole grafted seedlings have been examined at 8, 14, and 28 days after inoculations (Nishizawa and Ooyama 1981). No nematodes were recovered from resistant grafted seedlings 8 days after inoculation, but nearly 10 nematodes per g of wood were recovered from susceptible seedlings. Both susceptible P. thunbergii and resistant P. elliottii and P. massoniana had 10 to 10,000 nematodes per g of wood 28 days after inoculation, and subsequently died following additional inoculations. As a result of this work grafted seedlings used in resistance tests are now inoculated at least twice to assess their resistance to pine wood nematode.

Seminar on Pine Wilt Disease in the United States

On October 9, 1981 I gave a 100 minute seminar on distribution and research on pine wood nematode in the United States to 18 scientists at the Kyushu Branch Station. The seminar was similar to the one given at the central headquarters of the Forestry and Forest Products Research Institute. I discussed the pine wilt disease situation in the United States, the major locations where research on the pine wood nematode is being conducted,and indicated who was conducting the research. The scientists were especially interested in the hosts of the pine wood nematode, and major locations of P. densiflora and P. thunbergii in the United States. The scientists were also interested in work on control of Ceratocystis ulmi in the United States, and I discussed this work with them.

Field trip to Kyushu Forest Tree Breeding Institute

On October 9, 1981 I visited the Kyushu Forest Tree Breeding Institute, Forestry Agency, Ministry of Agriculture, Forestry and Fisheries with Dr. Yokata, Mr. Kiyohara, and Mr. Kawabe. The tree breeding institutes in Japan are located at Kyushu, Okayama, Kanto (Mito),Tohoku and Hokkaido. Dr. N. Ooyama and Mr. Kiyohara have current cooperative studies with the tree breeding institute at Kyushu. Dr. Yoshiyuki Fujimoto is chief of the section of forest tree

breeding. He is conducting a large breeding project for production of pine trees resistant to the pine wood nematode. This project is being carried out in cooperation with Kansai Forest Tree Breeding Institute, regional state forest offices and prefecture forest offices located in the geographical jurisdiction of the Kyushu Forest Tree Breeding Institute. The objective of the project is to develop pines with genetic resistance to pine wood nematode from pines that have survived in stands severely damaged by this nematode.

The general outline of the tree breeding project for resistance to the pine wood nematode is shown in Appendix 4. From 1978 to 1980, 25,000 Pinus densiflora and P. thunbergii trees were selected from severely damaged pine forests with less than 10% survival. Then 20 scions from each candidate tree are cleft grafted into 2-year-old P. thunbergii root stocks. In the second year after grafting, each of 10 grafted scions (ramets) from each candidate tree are artificially inoculated with 10,000 nematodes at 30 cm above the graft union. The first inoculations were initiated in 1980 when 4312 clones were inoculated (682 of the clones were tested at Kyushu). The bark from the lower portion of 2-year-old leader shoots of each scion was peeled back (1x5 cm), and 10,000 nematodes were dropped onto the peeled bark portion in a 0.1 ml water suspension. Tree survival is evaluated at 2-week intervals after inoculation by all cooperators, and at 8 weeks after inoculation all data is evaluated at the tree breeding institutes. Only 235 clones (192 P. densiflora and 43 P. thunbergii) or 5% of those clones tested passed the first inoculation (Appendix 5). All clones must survive and perform better than P. taeda scions grafted onto P. thunbergii stock to pass the inoculation test. Pinus taeda is used as the control in the resistance tests because it has appeared nearly immune to pine wood nematode in previous inoculation trials. P. taeda scions used as controls come from five seed trees of unknown provenance.

An additional 1209 clones were inoculated for the first time with pine wood nematode in 1981 at the Kyushu Tree Breeding Institute (Appendix 5), and only 11 clones or less than 1% survived the inoculation test. Dr. Fujimoto explained that high summer temperatures in the greenhouse (43 C) during the test period apparently contributed to this low survival percentage.

Clones that pass the first inoculation will be inoculated a second time (Appendix 4). In the second inoculation test, 20 grafted seedlings of each clone will be tested. The resistant pines finally chosen for use in outplanting in Japan will be progeny or cuttings from clones that survived the second inoculation test. Seed orchards consisting of resistant pines will be established. Dr. Fujimoto hopes to have more than 50 resistant pines of P. densiflora and P. thunbergii when inoculation tests are completed in 1984. By 1994, large quantities of seed should be available for use in regeneration programs. Dr. Fujimoto has indicated that some resistant clones may be propagated by grafting, and grafted trees planted on an emergency basis as necessary in national parks, seashore, and other plantations.

The nematodes used for inoculation of pines in the tree breeding project originally came from the Shimabara District in Nagasaki Prefecture. The 1977 nematode isolate was selected from a group of 18 isolates on the basis of its virulence. Pathogenicity tests made with this isolate prior to its use in resistance testing demonstrated that it is highly virulent; it killed 95% of all artificially inoculated P. thunbergii. Dr. Fujimoto indicated that the virulence of this nematode isolate is monitored annually prior to its use with test clones.

The procedure used by the tree breeding institute for mass cultivation of nematodes for inoculation purposes is illustrated in Appendix 6. About five billion nematodes are needed annually for inoculation tests at the Kyushu Forest Tree Breeding Station.

In summary, the approach used by the Kyushu Tree Breeding Institute for production of native pines resistant to the pine wood nematode is sound and appears to be a feasible means to control this nematode in Japan.

Kansai Branch Station
Forestry and Forest Products Research Institute
Momoyama-cho, Fushimi-km
Kyoto, 612 Japan
Visited October 12-16, 1981

I left Kumamoto during the morning of October 11 and arrived in Kyoto in early afternoon of the same day. I spent the weekend sightseeing in Kyoto, and visited the Kansai Branch Station on October 12-16, 1981.

Personnel of the Kansai Branch Station have responsibility for conducting research in silviculture (silviculture, forest management, soils, forest influences) and forest protection (forest pathology, forest entomology). The area covered by this branch station includes 14 Prefectures (Ishikawa, Fukui, Shiga,Mie, Wakayama, Nara, Osaka, Kyoto, Hyogo, Okayama, Hiroshima, Tottori, Shimane, and Yamaguchi). The station has been in operation for about 30 years.

There are nine staff members of the forest protection division. The organization of this division and estimates of staff members research time on pine wilt disease are as follows:

Dr. Haruyoshi Saho
Chief, Forest Protection Division, 10%

Forest Pathology Research

Mr.Shuji Kontani
Chief of Laboratory,50%

Dr. Kazuo Suzuki
Pathologist , 100%

Mr. Kazuhiko Mineo
Pathologist, 100%

Forest Entomology Research

Dr. Kazumi Kobayashi
Chief of Laboratory, 50%

Dr. Kensuke Ito
Entomologist, 50%

Mr. Motoo Okuda
Entomologist, 50%

Mr. Ryuji Hosoda
Entomologist, 50%

Mr. Kuwayhata
Wildlife Research

On October 12, 1981 I toured the laboratory with Dr. Saho, met staff members,and viewed facilities. The forest pathology section has one large preparatory laboratory which contains autoclaves, constant temperature water baths, incubators, walk-in coolers, and other general laboratory equipment. There are also two pathology labs that are well equipped to conduct histopathological and cultural work. Some equipment available to scientists includes a cryostat, sliding microtome, Leitz orthomat research microscopes, scanning electron microscope, and two laminar flow hoods. A gas chromatograph has been ordered for use in physiological work. In the entomology section there are three walk-in coolers and several growth chambers that are used to conduct temperature and photoperiod studies on forest insects, and a screen house that is used for conducting research on emergence of insects from infested logs. There are also four greenhouses, a nursery, an arboretum, and a demonstration forest (experimental plot) located on about 10 ha of land at the station. A 30 ha experimental site at Okayama is also available for conducting research. In general the laboratory, greenhouse, and other facilities appear very good for conducting forestry research.

I had discussions with forest pathologists and entomologists during my 5-day visit at the station,and their current research is as follows:

Mr. Shuji Kontani

Mr. S. Kontani, Chief of the Forest Pathology Laboraotry, is principally a mycologist but spends about 50% of his time on pine wilt disease. At present he is doing some research on chemical control of pine wilt disease. I did not have sufficient time to view or discuss his work during my visit to Kansai Branch Station.

Dr. Kazuo Suzuki--

Dr. Suzuki is a 1972 graduate of Tokyo University and has been associated with the Forestry and Forest Products Research Institute since 1973. He was stationed at Meguro from 1973 to 1974, at Kyushu Station from 1974 to 1979 where he did most of his research on pine wilt disease, at Shikoku Station from 1979 to 1981, and moved to Kansai Station in June 1981 when Mr. Tanaka was transferred to the Hokkaido Station. His doctorate research was on physiological aspects of Melamspora laricini-populina leaf rust of poplar. Because of his expertise and interest in physiological research, he continued this type of research at Kyushu and was one of the first investigators to study transporation and leaf water potentials in respect to pine wilt disease, and the relation between nematode populations and disease development (Suzuki and Kiyohara 1978, Kiyohara and Suzuki 1978). At Shikoku, he conducted research on discoloration and decay of Cryptomeria japonica (Suzuki 1981). From 1981 to 1984 he will be conducting physiological research on the mechanism of wilting in pine wilt disease. He will seek to answer questions such as why pines wilt after inoculation with B. xylophilus. Why do B. xylophilus populations increase when pines start to wilt? What triggers populations of B. xylophilus to increase rapidly? Are toxins involved in pine wilt, and are they the cause or result of pine wilt? He feels that more physiological data on the development of pine wilt disease is needed and will concentrate his efforts in this area.

Mr. Kazuhiko Mineo--

Mr. Mineo has a masters degree in forest pathology and does research full time on pine wood nematode. Prior to the discovery of the pine wood nematode he worked on plant-parasitic nematodes associated with roots of trees.

Mr. Mineo has inoculated several P. densiflora and P. thunbergii with B. xylophilus to determine the minimum number of nematodes needed to kill these pines in the Kansai region. This work is being conducted on the grounds at the branch station. In contrast to inoculation work at other locations in Japan, Mr. Mineo uses inoculum obtained from M. alternatus beetles because he feels such inoculum approaches natural conditions more than inoculum derived from fungus cultures on artificial medium. His 1980 and 1981 inoculations on P. thunbergii and P. densiflora include inoculum levels of 10,000, 5,000, 1,000, 500 and 100 nematodes. The levels used are based in part on previous laboratory work done at this station to determine how many nematodes are deposited at feeding sites on host stems. The average number of B. xylopilus carried by M. alternatus is 5,000. Researchers have estimated, by counting numbers of nematodes desposited on moistened filter paper in petri dishes, that 2,000 nematodes are deposited during a 24 hour feeding period. Mr. Mineo's inoculations typically have involved cutting outer bark into the wood, placing a wad of cotton on the wound, adding a 3-ml nematode suspension, and securing cotton in place with a plastic cover. His 1981 inoculations were made July 13 on terminal shoots, and on laterals and terminals of lateral branches of 6-year-old trees. Results of his inoculation test reveal that the minimum number of nematodes needed to kill each pine species was 100. At this inoculum level, 1 of 5 (20 percent) of the inoculated P. densiflora died and 3 of 5 (60 percent) of the P. thunbergii died when inoculations were made on tree terminals. Only 1 of 5 P. thunbergii died when the inoculations were made on lateral shoots. Tokushige and Kiyohara (1969) had reported earlier that 30 nematodes was the minimum number needed to kill native pines in Kyushu. Similarly, Kiyohara and Suzuki (1978) found that 50 nematodes could kill 3-

to 4-year-old P. thunbergii at the same location. Thus the current work on inoculum density needed to kill native pines by Mr. Mineo is a repeat of previous work but differs in that inoculum was recovered from Monochamus alternatus beetles.

In regard to the minimum number of nematodes needed to kill pines, Dr. Suzuki indicated that when pines are inoculated with B. xylophilus, normally only 20 to 30% of the initial inoculum remains alive at the inoculation site 1 to 2 days after inoculation (Dozono and Kiyohara 1976, Kiyohara and Suzuki 1978). Dr. Suzuki feels that high nematode mortality occurs in all artificial inoculations made with seedlings. More specific data on viability of nematodes following inoculations is available at the Kyushu Branch Station according to Dr. Suzuki.

Previous research at the Kansai Lab by Dr. N. Enda has provided basic information on the transmission of B. xylophilus by M. alternatus during maturation feeding. In one study conducted in 1973, 10 3-year-old seedlings were placed in screen cages containing a known number of recently emerged M. alternatus infested with B. xylophilus. These 10 seedlings were replaced with additional sets of 10 seedlings at weekly intervals for a period of 8 weeks. Results revealed that M. alternatus fed on many seedlings but transmitted nematodes only after the first week of feeding. Percent trees killed by transmitted nematodes during the second through eighth week were 70, 90, 80, 30, 50, 40, and 10 respectively.

Mr. Mineo is currently conducting a host range test on several outplanted tree species that he inoculated with 10,000 B. xylophilus. This work was started by Mr.Mineo and Mr. Tanaka before Mr. Tanaka moved to Hokkaido. Tree species that were not infected with B. xylophilus in the current test include

Cryptomeria japonica, Chamaecyparis obtusa, Abies firma, and Taxodium distichum. Many other trees species are now being grown at the station and will be used in future host range tests. Mr. Mineo has inoculated 20-year-old Pinus taeda (15 cm DBH and 9 m tall) twice with 50,000 B. xylophilus and they did not become infected. As a result of his host range tests, Mr. Mineo rates P. thunbergii and P. sylvestris as susceptible, P. luchuensis, P. nigra, densiflora, P. pinaster, P. massoniana, and P. elliotti as intermediate, and P. taeda, P. banksiana, and P. strobus as resistant to B. xylophilus. With the exception of P. luchuensis and P. pinaster, this host rating scheme is similar to the one reported by Nishizawa and Ooyama (1981) from Kyushu.

Dr. Haruyoshi Saho--

Dr. Saho discussed some of his research on pine wood nematode with me on October 12, 1981. Dr. Saho is a forest pathologist who has done extensive research on rusts of pines, principally in Hokkaido. He moved from Hokkaido to the Kansai Branch Station about 2 years ago, and I do not think he has had any formal training in nematology. He is still writing manuscripts on his rust research from Hokkaido. His work at Kansai is directed toward control of pine wood nematode in logs during winter months so that M. alternatus adults do not become infested with nematodes prior to their emergence the following spring. His approach to control of nematodes is to kill or eliminate their fungal food base with pesticides. He estimates that 6 months (November-May) is sufficient time to kill fungi in logs and "starve" nematodes so that M. alternatus does not become infested with nematodes prior to their emergence. Although Dr. Saho has tried a number of different types of pesticides, his best results have occurred with use of herbicides which kill fungi that colonize logs. The technique used is to drill holes (2 cm x 10-15 cm) placed 20 cm apart in logs,

then add 3 g sulfamic acid sodium salt per hole, and fill with 5% dimethyl sulfoxide. The dimethyl sulfoxide acts as a surfactant and aids in the movement of the sulfamic acid. The log is positioned so that boles will catch natural rain. It is not necessary to peel bark from the logs prior to treatment. He intends to continue this research to determine the minimum amount of chemical needed to kill colonizing fungi. Although this research provides information on killing of fungi in nematode infested logs, it does not appear to be a very effective means for ultimate control of pine wood nematode because most nematodes are in the third dispersal stage during winter months and do not feed on fungi.

Dr. H. Saho is also conducting work on biological control of pine wood nematode by inoculating nematode infested logs with a wood decay fungus (Polyporus versisporus) that is not a host for the nematodes. Logs are inoculated with P. versisporus in September, and the fungus rapidly invades recently killed logs and replaces fungal hosts (Botrytis, Ceratocystis, Fusarium) of the nematode. Within 1 year following inoculation nematodes are no longer present in the logs. The effectiveness of this research for the control of pine wood nematode must be evaluated carefully since the nematodes do not feed on fungi when in the third dispersal stage.

Dr. Kazumi Kobayashi--

Dr. Kobayashi is Chief of the Forest Entomology Lab. On October 15, 1981 I had a general discussion with him and staff entomologists on insect research being conducted at the Kansai Station. Dr. Kobayashi informed me that there are nine active studies, but most research at present is on Semanotus japonica/of beetle Cryptomeria and Monochamus alternatus beetle of Pinus. The research effort on M. alternatus is about 2 SY's per year.

The main project on M. alternatus is a 4-year study initiated in 1981 to determine how infestations develop. The study area is located at Omi-Hachiman which is near the southern border of Lake Biwa in Shiga Prefecture. Annually the scientists will photograph (aerial) the study area, analyze climatological data (temperature , rainfall), determine population dynamics of M. alternatus, and determine number of B. xylophilus in sawyers and in pine wood. This data will be used to develop a mathematical model for predicting how sawyer infestations fluctuate according to environmental conditions. Results of this work should also apply to infestations in southern Japan, including Shikoku in Kyushu.

Dr. K. Kobayashi informed me that he and his colleagues have accumulated considerable information on the biology of M. alternatus. The sawyers' feeding period is from early June through September with principal feeding occurring from mid-June to mid-August. The oviposition period extends from late June to early September. The flight of M. alternatus is random during maturation feeding, but beetles are attracted to dying pines during oviposition feeding. In most cases sawyers fly 500 m during feeding periods, but the longest recorded flight was 2 km.

Dr. Kobayashi discussed pine wilt disease development on the Noto Peninsula in northern Honshu,which demonstrates that sawyer flight is influenced by wind direction in July-August when adults are active. The direction of prevailing winds on the peninsula is from west to east. Mortality of pine caused by pine wood nematode was first observed on the west side (coastal area) of the peninsula in 1971, apparently from logs shipped to a seaport. The disease subsequently spread easterly to the central and northeast part of the Peninsula where pines were severely damaged. The disease has now reached Noto-Ogi (in the northeast part of the Peninsula), but has not spread southward to Ishikawa.

Mr. Motto Okuda--

Mr. Okuda discussed his work on emergence of M. alternatus from pine logs. He has collected pine infested with sawyers and placed them in a screen house. From these logs he is determining the number of M. alternatus that do and do not emerge per log, number of sawyers infested with B. xylophilus, number of B. xylophilus per sawyer, and predators and parasites of the sawyers. He has data on emergence of M. alternatus from 30 caged logs for a period of 8 years from 1973 to 1980. A summary of these data is as follows:

Year	Date of sawyer emergence from caged logs 1/			Total number adults emerged
	Initial	Peak	Final	
1973	6-15	7-5	7-30	781
1974	6-10	7-5	7-39	472
1975	6-5	7-5	7-25	798
1976	6-5	7-10	7-30	772
1977	6-5	6-30	7-30	509
1978	6-10	7-5	7-20	425
1979	6-15	6-25	7-25	510
1980	6-1	6-25	7-25	944

1/Pinus thunbergii logs, each 1.2 m long.

He found that the number of M. alternatus emerging from caged logs is dependent upon average day temperature and surface area of the logs. At temperatures of 12.5 C M. alternatus adults do not emerge. He and other scientists determined that a threshold temperature of 11.5 C is needed for M. alternatus to develop. Some 500 days accumulative temperature above 12.5 C is needed for peak sawyer emergence from the overwintering larval stage. By using this information, these scientists can predict when sawyers will emerge. This prediction is very important for timing of application of insecticides for control of the sawyers. Emergence of sawyers starts at 300 day accumulative temperature above 12.5 C, and terminates at 700 days accumulative temperature above 12.5 C.

The number of sawyers emerging from caged logs is expressed as number of sawyers per m^2 of wood surface. The average number of M. alternatus emerging per m^2 surface from caged pine logs is 20 to 30, with a maximum (physical limit) of 50 from P. densiflora and P. thunbergii. Although natural enemies (parasites and predators) do kill M. alternatus, Mr. Okuda indicated that the main factor controlling population levels is the number of newly dying trees that attract sawyers for oviposition.

Dr. Kensuke Ito--

Dr. Ito is conducting research on rearing of M. alternatus and Semanotus japonica beetles on artificial diets. Dr. Ito graduated from Department of Zoology, University of Hokkaido in 1980. He then spent approximately 1 year at the central headquarters of the Institute before moving to Kansai Station in April 1981. He indicated that about 10 years ago Dr. Yamane started initial research on artificial cultivation of M. alternatus, but the research apparently was abandoned for a 5-year period. The research was recently reactivated, and entomologists have succeeded in artificial rearing of M. alternatus from first instar larvae through the adult stage.

The diet that Mr. Ito is using to rear M. alternatus is as follows:

Ingredient	Amount
Pinus thunbergii dried inner bark	200 g
P. thunbergii dried wood	100 g
Agar	40 g
Yeast	40 g
Sodium propionate	1.0 g
Dehydroacetic acid, sodium salt	0.3 g
Ascorbic acid	3.0 g
Water	1000 ml

The agar is boiled in the water until melted, then the remaining ingredients are added and thoroughly mixed. The mixture is then dispensed in 60 ml plastic cups which contains enough diet to rear M. alternatus larvae to adults.

The artificial rearing of M. alternatus provides the technology necessary to obtain additional basic information on its biology. Work on temperature relationships to sawyer development can now be refined, and Mr. Ito has already started work on effects of photoperiodism on larval development. Another aspect of M. alternatus development that will be investigated is the mechanism involved in pupation. Completion of this basic work will provide additional information to refine predictions of when beetles emerge from infected trees.

Mr. Ryuji Hosodo--

Mr. R. Hosoda's principal research responsibility during the next 2 to 3 years is to investigate the association of M. alternatus and B. xylophilus. This new area of research includes evaluation of B. xylophilus populations in M. alternatus beetles, and factors contributing to fluctuations of beetle and nematode populations.

Seminar on Pine Wilt Disease in the United States

On October 12, 1981 I gave a 75 minute seminar on distribution and research on the pine wilt disease in the United States to 11 scientists at the Kansai Branch Station. Some of the scientists indicated that the late winter late spring pine wilt mortality as reported by Dr. R.Malek in Illinois also occurs in Shikoku and in some southern parts of the Kansai region. Many scientists were interested in the Bursaphelenchus species that Massey(1974) found associated with bark beetles in the United States.

Field trips in the Kansai Region

On October 13, Dr. Saho, Mr. Mineo, and I went on a field trip to Nara and surrounding area to observe pine wilt. The first mortality from pine wood nematode in this area was observed 17 years ago (1964) at a seaport near the Pacific Ocean. We visited Nara Park and observed occasional mortality of

P. densiflora. Dr. Saho said that the most important pine trees in the park are sprayed with insecticide, usually in June and July, to prevent maturation feeding by M. alternatus. However, all pines in the park are not sprayed because of cost. We also observed an area in the park where a 90-year-old P. densiflora tree was recently cut. Many valuable pine trees had died in this small area, and it appeared to be a good location to investigate local spread of pine wilt disease by root grafting. Spread of pine wilt disease by root grafting has not been demonstrated to my knowledge.

I observed very extensive mortality of natural regeneration of P. densiflora at three locations on slopes near Takamadoyama Mountain southeast of Nara. The mortality was occurring on private land where control measures were not taken to prevent spread of the disease. Mortality of 30-year-old and younger pines was common since the older dominant trees were already killed . Dr. Saho indicated that pine mortality has been severe for the last 3 years in this area and suggests that all pines at these locations may be killed in another 3 to 5 years. Maturation feeding in many areas of Japan apparently occurs most often on older dominant trees, and then on younger trees when the dominants are killed. This pattern of pine mortality contrasts with that as experienced in the United States, where intermediate and suppressed trees are the first to be killed in many instances.

On October 14, 1981, Dr. Saho, Dr. Suzuki, and I made a field trip north of Kyoto and west of Omi-Imazu (in Shiga Prefecture) where we visited the Asahi Forest Environment Institute. This Institute which is owned by Asahi Newspaper Company, was established in 1980,and occupies an area of 150 ha. The chief research officer is Mr. Katsuhiko Shamada, who is an ecologist with a masters degree from Tokyo University. He is currently working on a floristic study of the Institute lands, and has recently completed a cover type map of the area. He has designated areas for planting and selected species to be planted based on the current local flora. The Forestry and Forest Products Research Institute

does not have any cooperative studies with the Asahi Forest Environment Institute at the present time.

During a tour of some pine stands in the southeast portion of the forest, we observed an area where pine wilt disease was initially discovered in 1978 on the property of the Asahi Institute. Two Pinus densiflora trees over 50-years-old were initially infected by pine wood nematode during the hot and dry 1978 growing season. Institute personnel have not made any attempt to control the disease because of expenses involved, and it has spread to new areas. We observed two areas where less than 10 P. densiflora were dying following infection in 1981. It appears that additional pine mortality could be avoided in the forest by prompt removal and destruction of the dying trees before the vector emerges in June 1982.

Laboratory of Plant Pathology
Department of Agronomy
College of Agriculture
Okayama University
Tsushima, Okayama 700, Japan
Visited October 16, 1981

On October 16, 1981 I visited with Dr. Hachiro Oku and Dr. Seiji Ouchi of Okayama University for approximately 6 hours. Dr. Oku is professor and Dr. Ouchi is assistant professor of plant pathology. The laboratory of plant pathology is one of six laboratories of the Department of Agronomy, College of Agriculture. There are three staff members for each laboratory, a professor, an associate professor, and an assistant professor (Dr. T. Shiraishi is assistant professor of the plant pathology laboratory). There is one university senior student working in the laboratory who has been accepted by the the graduate school and will soon start graduate study in plant pathology. Dr. Oku informed me that students may earn a Masters degree in this laboratory, but not a doctorate degree. Dr. Oku indicated that only eight institutions offer an advanced graduate degree in Japan: Universities of Tokyo, Hokkaido, Kyushu, Tohoku, Nagoya, Kyoto, Osaka Prefecture University, and Agricultural University of Tokyo (a private university).

Dr. Oku teaches two courses every year and his remaining time is spent conducting research. He has conducted research on host parasite relations of powdery mildews of barley and wheat, and spends about one third of his time doing research on toxins and mechanism of wilting in pine wilt disease. This work is supported by the Ministry of Education and Science, not the Ministry of Agriculture and Fisheries. Most of his research apparently is done in one large laboratory which is well equipped and includes two recently purchased high pressure liquid chromatography units. There is a forest located on University property where experimental field work is conducted.

Dr. Oku and colleagues have published three articles on toxins associated with pine wilt disease (Oku et al 1979, Oku et al 1980, Oku et al 1981), but have not established experimentally that toxins are involved in pine wilt disease. Dr. Oku indicated that their published work on toxins was done using thin layer chromatography (TLC) for isolation of toxins in artificially inoculated and naturally infected pines. Some new results have been obtained using high pressure liquid chromatography (HPLC). Therefore he is delaying further publication of research until he gets more information on the structure of toxins he has isolated.

The basic hypothesis for the involvement of toxins in pine wilt disease is supported by observations made by several investigations during the last 5 years. Histological and physiological work has established that destruction of epithelial cells of resin canals is associated with rapid wilting of pines (Mamiya 1975, Suzuki and Kiyohara 1978). However, Mamiya has found that discoloration of epithelial cells of resin canals occurs prior to invasion by B. xylophilus. These results suggest that some factor at the molecular level might be involved in destruction of the epithelial cells.

In their initial research, Oku et al (1979, 1980) isolated a toxin from artificially inoculated 3-year-old P. thunbergii by TLC. He applied a toxin to a 3-year-old P. densiflora, and it began to wilt within 14 days. A 1-month-old seedling similarly wilted in 16 hours after application of the toxin. They called this toxin 3L. This toxin apparently destroyed parenchyma and epithelial cells, and finally the entire seedling wilted. They detected 3L from naturally infected and nematode inoculated pines in 1978 and 1979. This toxin was not found in 1980, but another toxin with a Rf value (TLC) different than that of 3L was isolated. The reason for differences in production of toxin between 1979

and 1980 is not known, but Dr. Oku thinks that a difference in summer climate in 1980 (abnormally cool and rainy) caused a qualitative change of toxin precursor (Oku et al 1981). In 1981 Dr. Oku used HPLC and has isolated at least nine toxins from leaf extracts from inoculated seedlings (Appendix 7), including the finding of 3L in healthy control trees. He indicated that the substance from healthy trees is basic and cannot be extracted with organic solvents of acidic reaction.

Dr. Oku has produced the main toxin (3L) in pine leaf juice by inoculation with B. xylophilus or with a bacterium (a Pseudomonas species) that is commonly found associated with B. xylophilus. He has detected toxin as early as 5 days after inoculation of pine leaf juice with nematodes. Maximum toxic production occurred 15 days after inoculation. In our conversation Dr. Oku indicated that he was not certain that the toxin produced in vitro on pine leaf juice by the bacterium (Appendix 8), and that obtained from an inoculated 3-year-old pine seedling by HPLC are the same.

Dr. Oku and his colleagues have also found catechol and large quantities of benzoic acid in diseased pine leaves (Oku et al 1981). The concentration of benzoic acid sometimes reaches concentrations of more than 1,000 ppm (dry weight basis) at the early stage of wilting in naturally infected pine leaves. Healthy pine leaves do not contain this acid, but senescent or naturally dead pine leaves have about 100 ppm benzoic acid. This acid also accumulates in pine leaves of 2-year-old seedlings 14 days after inoculation with 4,000 B. xylophilus. Some 35 days after inoculation 1981 leaves had 148 ppm (dry weight basis), while 1980 leaves had 855 ppm (Appendix 9).

Results of Dr. Oku's work show that substances from pine wood are toxic to pine seedlings, but that it is difficult to get experimental proof of their involvement in pine wilt disease. Some scientists have been critical of Dr. Oku's research and feel that he published results before repeating his work. Dr. Oku did indicate that his work on pine wilt disease represented the first time he used trees as experimental plants. Dr. Oku said that he plans to come to the United States for a 3-month period from mid-April 1982 to mid-July 1982 to study toxins of pine wilt disease with scientists at the University of Missouri at Columbia and St. Louis.

In the afternoon of October 16, 1981, Dr. Oku and Dr. Ouchi took me to the northeast part of Okayama where I viewed very severe pine wilt damage to P. densiflora on a mountain slope in the Tatsunokuchi area. The pine mortality was occurring on national forest land that was last sprayed in 1978 to control M. alternatus. The sawyers were controlled by aerial spraying with a phosphorus compound in 1976, 1977, and 1978. After 1978 the local citizens objected to the spraying because they felt the spraying killed off natural enemies of the sawyer as well as honey bees and birds. The spraying for control of the vector was subsequently stopped. The severe pine damage that has occurred in this area may contribute to a serious erosion problem on the hillsides in the future.

In the evening of October 16, I took a train to Tokyo, and spent the weekend shopping (October 17 with Dr. Mamiya) and relaxing.

Tohoku Branch Station
Forestry and Forest Products Research Institute
Nabeyashiki, Shimokuriyagawa
Morioka, 020-01, Japan
Visited October 20-22, 1981

I visited the Tohoku Branch Station with Dr. Y. Mamiya during the week of October 19-24, 1981. The Tohoku District is located in the northern part of Honshu and consists of six prefecture (Aomori, Akita, Iwate, Yamagata, Miyagi and Fukushima). The total area is 6,689,000 ha, of which 1,208,000 ha (18%) is agricultural land and 4,704,000 ha (80%) is forest. Ownership of forest is 44% national, 8% local government, and 48% private.

There are high mountains running from north to south in the central portion of Tohoku. The climate of the district is separated into two types by the mountains. One climate is the Japan Sea type where annual precipitation is over 1,500 mm and snow depth is about 100 cm at the forest facing the Japan Sea. The other climate is the Pacific Ocean type where less than 1,300 mm annual precipitation occurs in the mountain area. The annual average temperature is more than 11 C in the Japan Sea type, and it is colder in the Pacific Ocean type.

Forestry research at the branch station is being conducted in areas of silviculture (two laboratories), management (two laboratories), soils, influences, forest pathology, forest entomology, and wildlife. The forest pathology project conducts research on diseases of nursery stock, diseases of conifers (Larix species, Pinus pentaphylla, P. pumila, Cryptomeria japonica), witches' broom of cherry trees, and the biology of the pine wood nematode.

The organization of the forest protection section is as follows:

Dr. Higachi
Chief of Forest Protection Section

Forest Pathology Res.	Forest Entomology Research	Wildlife Research
Dr. Yoshiyuki Zinno	Mr. Takizawa	Dr. Yui
Chief of Laboratory	Chief of Laboratory	Chief of Laboratory
Mr. Tsugio Shôji	Mr. Igarashi	Mr. Suzuki
Pathologist	Entomologist	
Mr. Yoshinori Yokozawa	Mr. Yannbe	
Pathologist	Entomologist	

The purpose of the visit to the Tohoku Station was to observe measures used to prevent spread of pine wilt disease into healthy pine stands. Pine wilt disease now occurs in Iwate, Yamagata, Miyagi, and Fukushima Prefectures in the Tohoku District. It was found in Ishinomaki (Miyagi Prefecture) in 1975 near a seaport, and in Morioka (Iwate Prefecture) in 1979. These infection centers were started through movement of logs infested with M. alternatus and B. xylophilus from other prefectures. These infection centers have provided a good opportunity to control spread of the disease by felling and removing infected trees before the sawyers emerge.

The summer temperatures in the district are sufficient for development of pine wilt disease, but the summers are shorter than in the southern areas of Japan. Many of the pines are larger than in other areas of Japan and are valuable for lumber and pulp. Thus there is much interest in preventing spread of pine wilt disease.

In 1982, the forest pathology lab will officially join the 1981-1984 Institute-wide pine wood nematode project. At the present time Dr. Zinno spends about 30% of his research time, and Mr. Shôji about 50% of his time on pine wilt disease. Their research is directed toward determining why pine wilt disease is not common nor spreading rapidly in the district. They are examining vector distribution and environmental conditions to evaluate how pine wilt disease develops in northern Japan. They want to be able to predict if the disease will spread into northern areas from newly established infection centers. The present distribution of Monochamus alternatus is wider than the distribution of the pine wilt disease in the Tohoku District, and Mr. Shôji (1979) has already established that B. xylophilus can survive in the northern part of Japan. At the present time it does not appear that disease development will be similar to that in southern prefectures like Ibaraki. Dr. Zinno and Mr. Shôji cannot conduct inoculation tests with pine wood nematode in the Tohoku District because dying and dead pines are removed within a very short time by personnel of the prefecture government. In this district the prefecture government is in charge of cutting, removal, and burning of diseased trees.

Seminar in Pine Wilt Disease in the United States

On October 22, 1981, I gave a seminar to 22 scientists at the Tohoku Branch Station. During the seminar I discussed distribution and research on pine wilt disease in the United States. I also explained how the USDA Forest Service research branch was organized and how research is conducted in general. Dr. Mamiya kindly translated the presentation into Japanese for the attending scientists. Following the seminar I had an enjoyable evening meal with Director Takashi Ito, Dr. Zinno, Dr. Mamiya, Dr. Higachi, Mr. Shôji, and Mr. Yanagiya who is the research coordinator of the Tohoku Branch Station.

Field trip to Akita and Aomori Prefectures

On October 20-21, 1981, I took a field trip with Mr. Shôji and Dr. Y. Mamiya through Akita and Aomori Prefectures, an area where pine wilt disease does not occur. Lack of pine wilt disease in these prefectures may be due to lower temperatures and a shorter growing season than in southern areas of Japan. Approximately 15 miles north of Morioka we observed a 110-year-old natural stand of P. densiflora. This 8.4 ha stand is now an experimental forest which has been reserved for research. Most of this area supported similar natural stands in the past but many were cut for lumber during the war. At present there are 360,000 ha of P. densiflora and P. thunbergii, 200,000 ha of Larix, and 1,000,000 ha of Cryptomeria japonica in the Tohoku District.

A number of diseases of forest trees was observed during the field trip. At Matsukana we observed larch shoot blight, Guignardia laricina, on Larix leptolepis (Sato et al 1963). Other hosts include L. occidentalis and L. laricina. Some 68,000 ha of larch plantations are infected by this pathogen in the Tohoku District. When most of the trees growing shoots become infected, growth essentially stops and many branch brooms can be seen in the crown. This pathogen is initially spread by infected nursery stock, and in the forest it is spread by ascospores which are dispersed in June. The pathogen is controlled in the nursery by application of fungicides. The most effective control of the pathogen in forests, according to Mr. Shôji, is to fell infected trees and burn them.

In Akita Prefecture near Hanawa we observed a canker disease of Paulownia tomentosa that is caused by Valsa paulowniae. Wood from this tree species is used for making furniture, and trees must be pruned to improve wood quality. However, the pathogen enters through pruning wounds, causes large cankers, and thereby reduces wood quality. The pathogen can be controlled by applying benomyl to pruned branch surfaces. Mr. Shôji is just starting research on this disease, and will be conducting work on factors affecting disease development.

At Yakeyama in Aomori Prefecture we observed a foliage dieback of Cryptomeria japonica. This disease occurs throughout Japan. Mr. Shôji and Mr. Yokozawa have studied this disease for many years but still have not determined the causal organism. The foliage of branches in the crown dies following infection and girdling of stems. Numerous fruiting bodies were observed on infected stems, but their spores will not germinate when placed on a variety of cultural media. Mr. Shôji has isolated a fungus from mycelia recovered from surfaces of infected stems but fruiting bodies do not develop in culture. Typical disease symptoms develop when trees are inoculated with the isolated fungus.

Field trip to Iwate Prefecture

On October 22, 1981 I took a field trip to Ichinoseki in Iwate Prefecture with Dr. Zinno, Dr. Mamiya, and Mr. Takeshi Sakuyama who is the forest pathologist for the Iwate Prefecture Government. His official station is in Morioka. He has worked on Lophoderium needle cast of pines and control of the causal fungus in nurseries. He has also investigated the ecology and control of Eastern gall rust (Cronartium quercuum) on P. densiflora (Sakuyamu 1979). At the branch office of the Prefecture Government in Ichinoseki, we met Mr. Tsukasa Kamata, a forestry specialist. Mr. Kamta is monitoring the pine wilt disease in Ichinoseki. He samples dying pine trees, sends samples to Mr. Sakuyama for confirmation of presence of pine wood nematodes, and has all infected trees removed and destroyed. The Prefective Government is responsible for control of pine wilt disease; their current policy is to fell, remove, and burn dying and dead pine trees to protect their pine resource (Appendix 10). They provide funds and labor to remove infected trees and have encountered little resistance from private landowners on removal of infected trees.

The occurrence of pine wilt disease on P. densiflora in Ichinoseki during the past 3 years is summarized below:

Date	Dead trees (no.)	Volume dead (m^3)	Trees sampled (no.)	Trees with pine wilt disease (no.)	(%)
1979	766	196.3	266	33	12.4
1980	482	66.1	92	19	20.7
1981	149	43.6	85	16	18.8

The percent occurrence of pine wood nematodes in trees sampled in 1979-1981 appears low, but in cooler areas nematodes are not found in all parts of the tree and recovery of pine wood nematode depends on sampling methods used. Mr. Kamata indicated that some affected trees exhibited flagging in the fall and trees died the following spring. This situation is similar to disease development that occurs in Illinois as reported by Malek and McClary (1981).

We examined dead pine trees in three areas in Ichinoseki. Near a sawmill, we observed a 60-year-old P. densiflora tree that had recently died. Two other dead pines were observed on a hillside near the sawmill. Possibly the source of M. alternatus and B. xylophilus may have been pine logs located at the sawmill. Pine logs are brought to the sawmill from various locations in Japan, but whether any logs came from pine wood nematode infected areas such as Ibaraki Prefecture is not known. In 1979, the owner of the sawmill at Ishinomaki in Miyagi Prefecture (directly south of Iwate Prefecture) admitted that some logs used for pulp came from Ibaraki Prefecture. It is believed that these logs contained the pine sawyer and pine wood nematode and started the infection center at Ishinomaki. At that time the owner of the sawmill removed dead pine trees from the area surrounding the sawmill. However, the owner no longer removes dead pines.

At the second stop a declining P. densiflora near a residence still had green foliage in portions of the crown. Mr. Sakuyama and Mr. Kamata indicated that such symptom development was typical of the cooler areas of Japan. The foliage symptoms were similar to those on pine of the late winter late spring mortality category as reported by Malek and McClary (1981). At this location another dead pine tree was observed in the stand on a ridge behind the residence. These trees will be removed and burned as soon as possible.

The pine wilt disease control program in Miyagi Prefecture is basically the same as practiced in Iwate Prefecture.

In theory the policy of felling and immediately burning dying and dead pine trees should be effective but its success is dependent upon quick removal and destruction of all infected trees. In practice it is virtually impossible to locate and remove all pine wood nematode infected trees with M. alternatus before the sawyers emerge and feed on healthy trees in the spring. Also in the overall control program in Iwate Prefecture and other prefectures there is an apparent lack of rigid enforcement of laws pertaining to movement of infected logs to noninfected areas by owners of sawmills of by homeowners. Movement of infested logs may lead to establishment of new infection centers in noninfested areas.

On the return trip to Morioka, we observed Rhizina undulata root rot of P. densiflora in a campground near the city of Kitakami. Dr. K. Ito in 1968 first described Rhizina root rot of pine trees from materials collected at Ishinomaki in Miyagi prefecture. We observed dying of P. densiflora in small groups associated with campfire sites. The high soil temperatures associated with fires (40 C) stimulates germination of ascospores of R. undulata and apparently kills many other soil fungi. Dr. Zinno is working on the biology and control of R. undulata. He has used pine stems placed at the margins of infection centers to monitor spread of the fungus. The fungus infects the stems and forms rhizomorphs (rhizoids) in the cambial area that are easy to identify.

He has indicated that the fungus spreads outward from infection centers at the rate of 3 to 4 m per year. A more detailed account of this disease has been published by Sato et al (1974).

There is no association between R. undulata root rot and pine wilt disease. Trees weakened by this fungus may attract many kinds of insects.

Pine wilt disease control programs used in some locations in the United States involve felling and burning infected trees. Since infections by this fungus originate from high temperatures associated with burning slash, campfires or bonfires, it is not advisable to recommend burning infected trees near or bordering residual pine stands.

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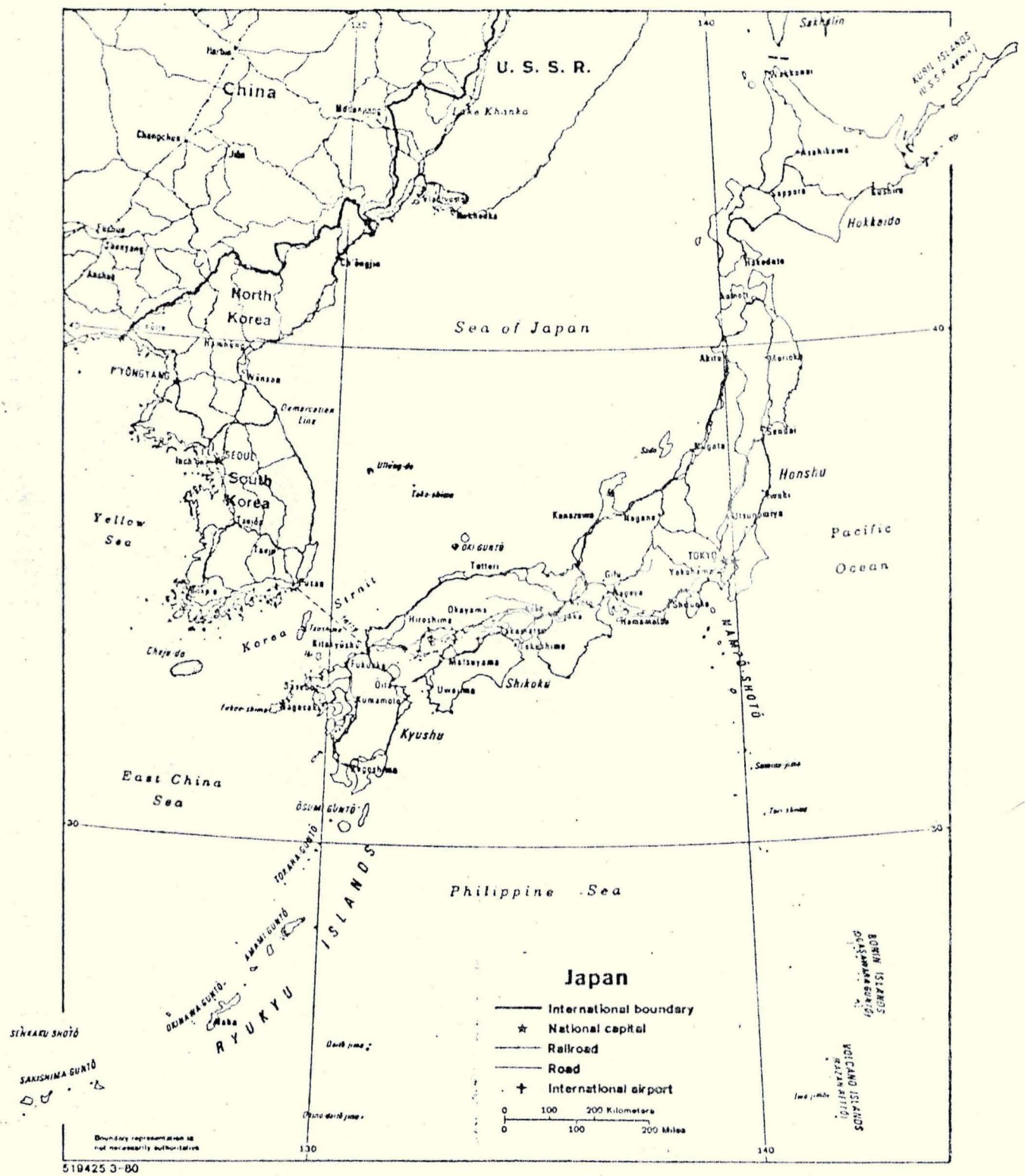
U. S. S. R.
China
North Korea
South Korea
Sea of Japan
Yellow Sea
East China Sea
Philippine Sea
Pacific Ocean
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Kyushu
Sakhalin
PYÖNGYANG
SEOUL
TOKYO
Korea Strait
RYUKYU ISLANDS
Japan
International boundary
National capital
Railroad
Road
International airport
0 100 200 Kilometers
0 100 200 Miles
Boundary representation is not necessarily authoritative
519425 3-80

ITINERARY

Dr. J. W. Riffle; Rocky Mountain Forest and Range Expt. Stn., USDA,Forest Service
September 29- October 28, 1981

September
29 (Tue) Ar. New Tokyo International Airport (Narita)
Accommodation: Tsukuba (Government Housing) Sept. 29-Oct. 4

30 (Wed) October 3(Sat)
Visit : Forestry and Forest Products Research Institute and pine wilt area in Ibaraki Pref.

October 4(Sun)
5(Mon) Lv. Tsukuba Ar. Tokyo Lv. Tokyo Ar. Oita by airplane TOA flight 333
Visit: Pine wilt area in Oita Pref. (Kyushu)
Accommodation: Beppu in Oita Pref. (Hotel)
6 (Tues) Visit: Pine wilt area in Oita Pref.
Accommodation: Kumamota Hotels Oct. 6-9

7(Wed)-8(Thur) Visit: Kyushu Branch of FFPRI

9(Fri) Visit: Kyusku Tree Breeing Institute in Kumamoto Pref.

10(Sat) Lv. Kumamoto Ar. Kyoto via Osaka by airplane (ANA Flight 524)
Accommodation: Kyoto Grand Hotel Oct.10-15

11(Sun) Stay in Kyoto

12 (Mon) Visit:Kansai Branch of FFPRI

13(Tues) Visit pine wilt area around Nara City

14(Wed) Visit pine wilt area in Shiga Prefecture, and Asahi Forest Environment Inst

15(Thur)Visit Kyushu Branch of FFPRI

16(Fri):Lv.Kyoto,Ar. Okayama by train
Visit: Okayama University & pine wilt area with Dr. Oku and Dr. Ouchi

Lv.Okayama, Ar. Tokyo
Accommodation:Tokyo (Hotel) Oct. 16-18

19(Mon) Lv. Tokyo, Ar. Morioka by train
Accommodation: Morioka (Hotel)

20(Tue) Lv. Morioka, Ar. Towada in Aomori Pref. by car
Accommodation: Towada Grand Hotel

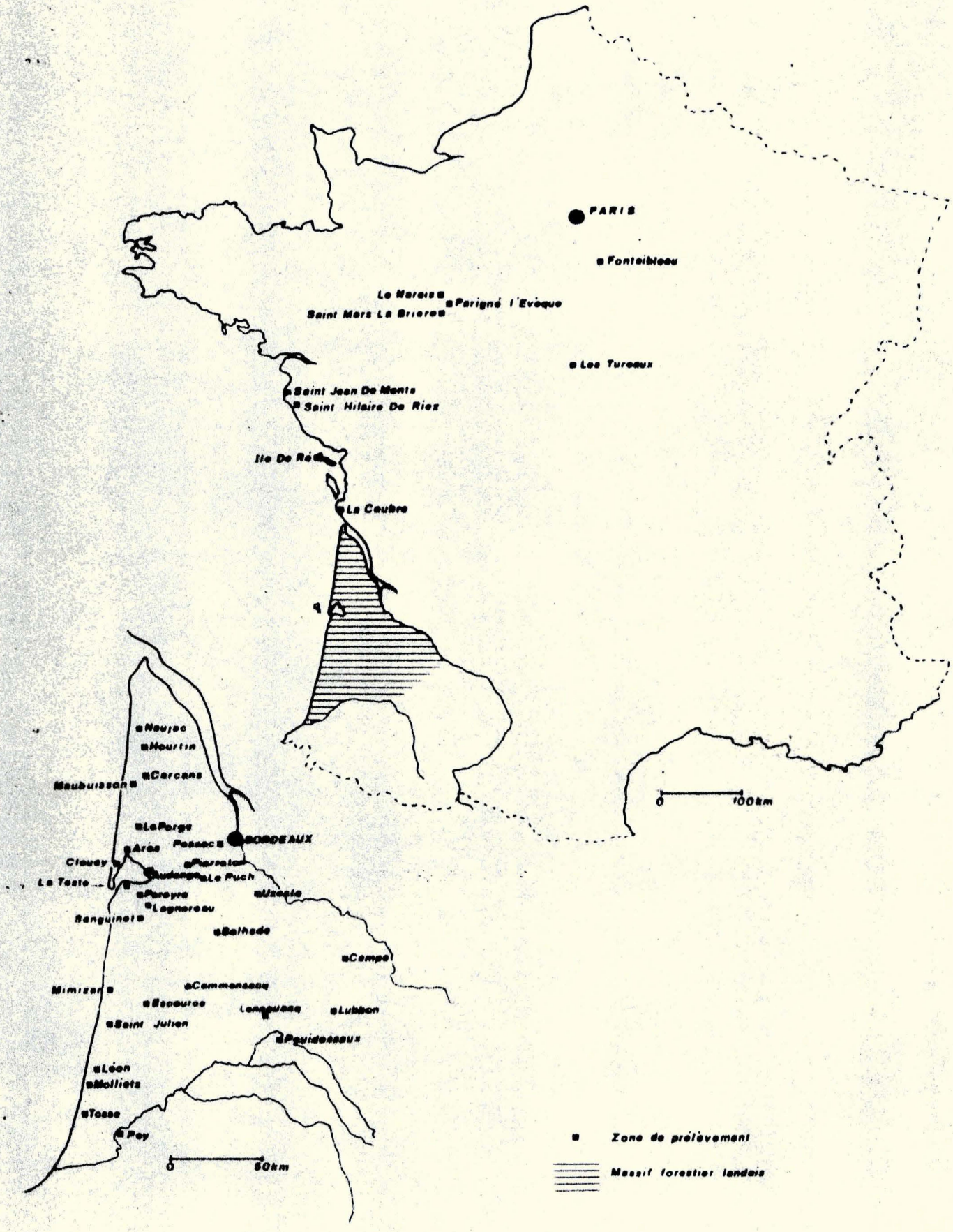
21(Wed) Lv. Towada, Ar. Morioka by car
Accommodation: Morioka Hotel (Oct. 21-22)

22(Thur) Observe pine wilt in Ichinoseki (Awate Pref.), and visit Tohoku Branch of FFPRI

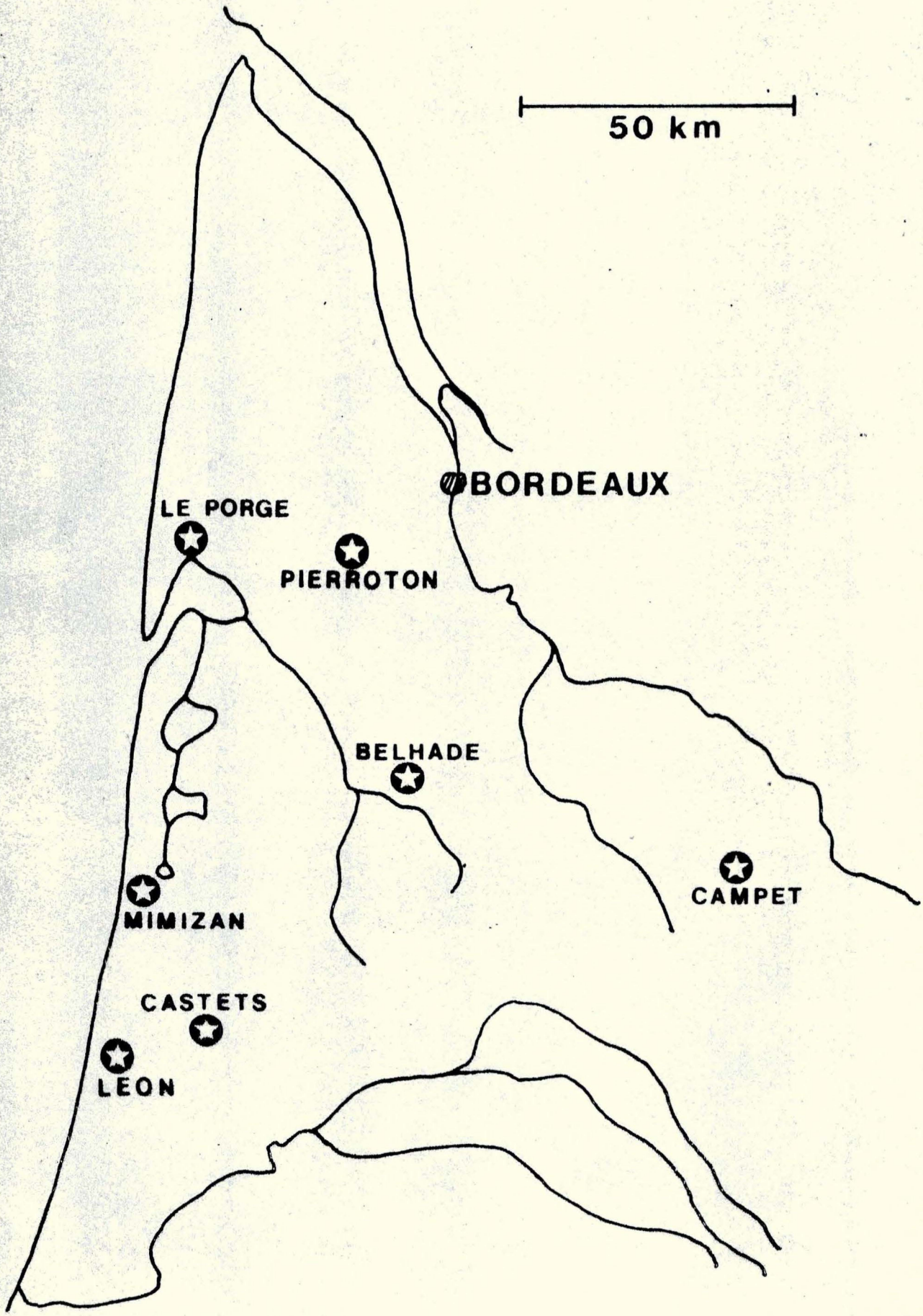
23(Fri) Lv. Morioka Ar. Tsukuba by train
Accommodation: Tsukuba (Government Housing) Oct. 23-27

24(Sat)-27(Tue)Visit: FFPRI

28(Wed) Lv. New Tokyo International Airport (Narita)


PARIS
Fontainbleau
Le Marais
Perigné l'Evêque
Saint Mars La Brieres
Les Tureaux
Saint Jean De Monts
Saint Hilaire De Riez
Ile De Ré
La Coubre
Naujac
Hourtin
Carcans
Maubuisson
LaPorge
Arès
Pessac
BORDEAUX
Cleucy
Le Teste
Le Puch
Parcyre
Lagnereau
Sanguinet
Salhade
Campet
Mimizan
Commensacq
Escource
Lubbon
Saint Julien
Pouydesseaux
Léon
Molliets
Tosse
Pey
0 100km
0 50km
Zone de prélèvement
Massif forestier landais

Bursaphelenchus lignicolus in France



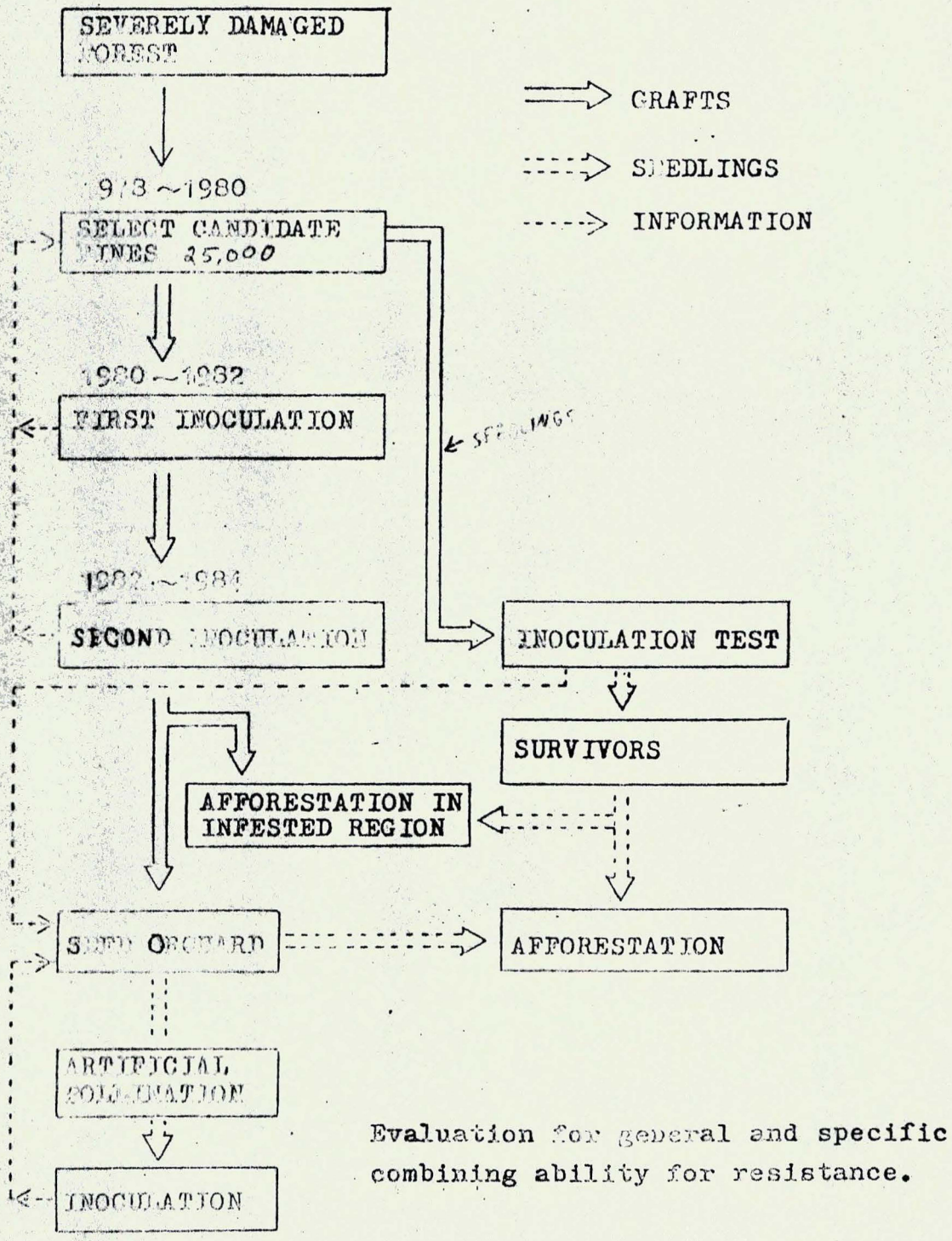


Figure . The program of the breeding project of the Japanese pines for resistance to the wood nematode.

Kyushu Forest Tree Breeding Institute

Tree Breeding for Resistance to Bursaphelenchus xylophilus

Results of the first inoculation

1980 (17 organizations)

	inoculated(clones)	passed(clones)	%
P.densiflora	1768	192	11
P.thunbergii	2544	43	2
Total	4312	235	5.4

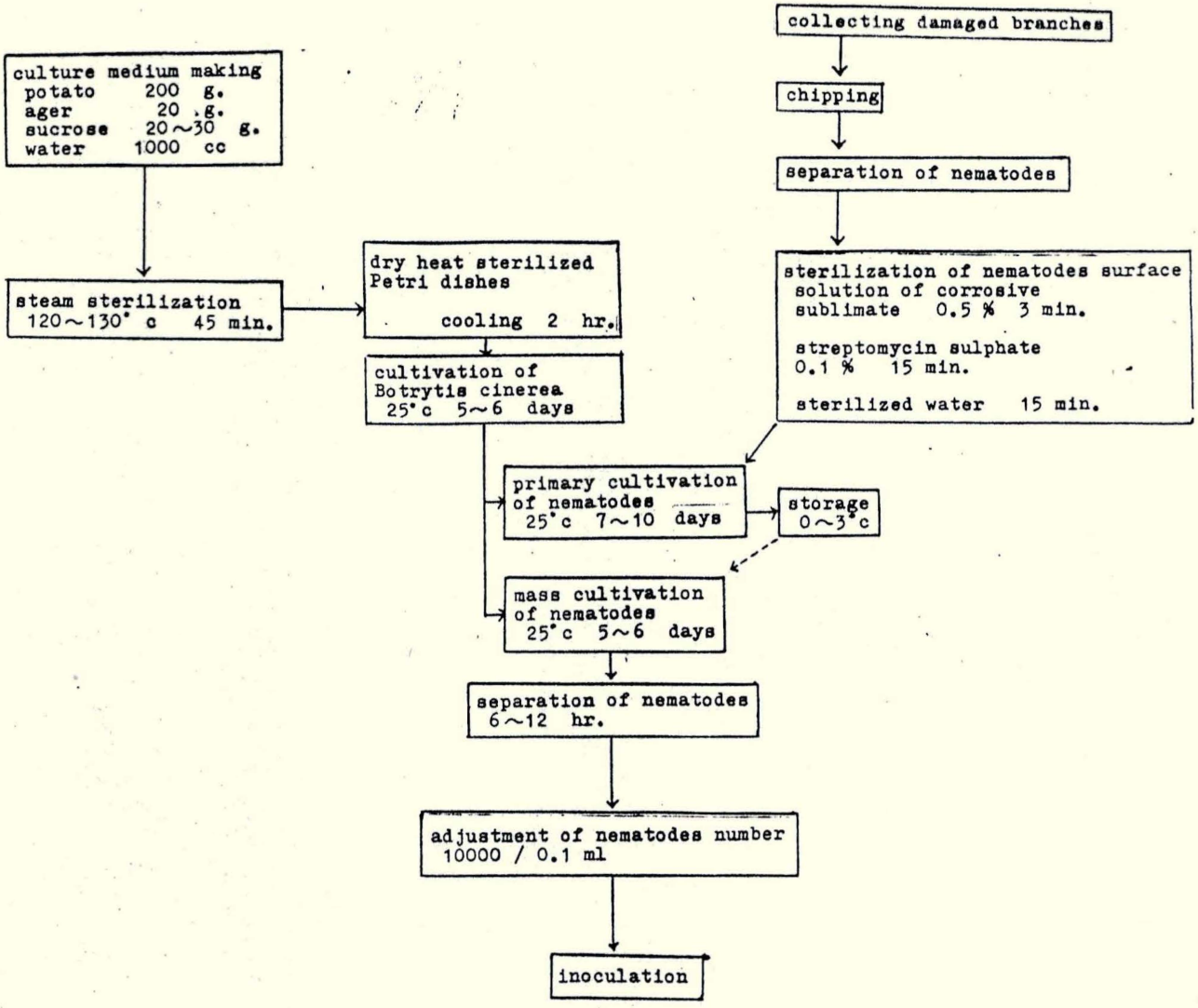
1980 (Kyushu Forest Tree Breeding Institute)

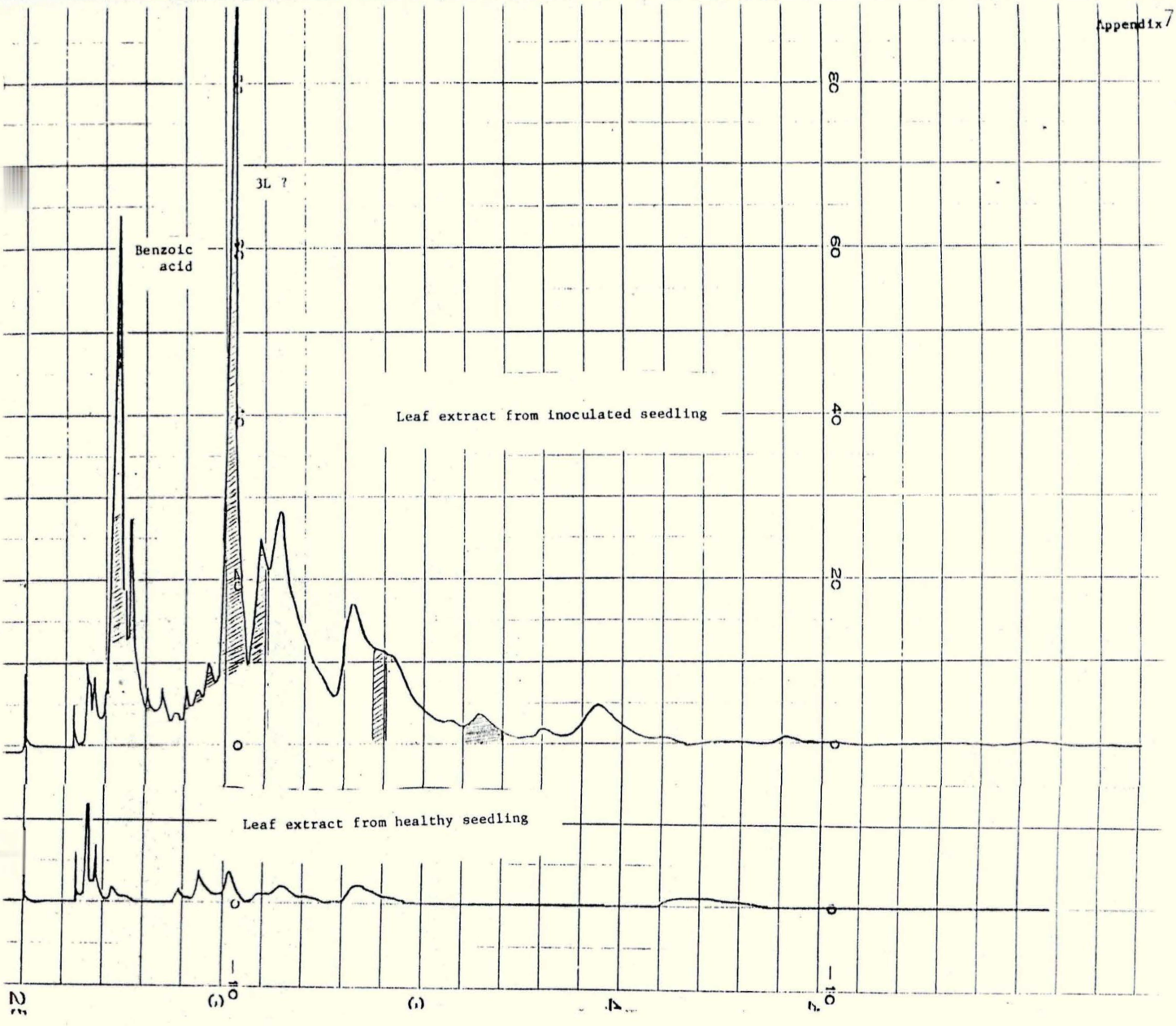
P.densiflora	283	30	11
P.thunbergii	399	2	0.5
Total	682	32	4.7

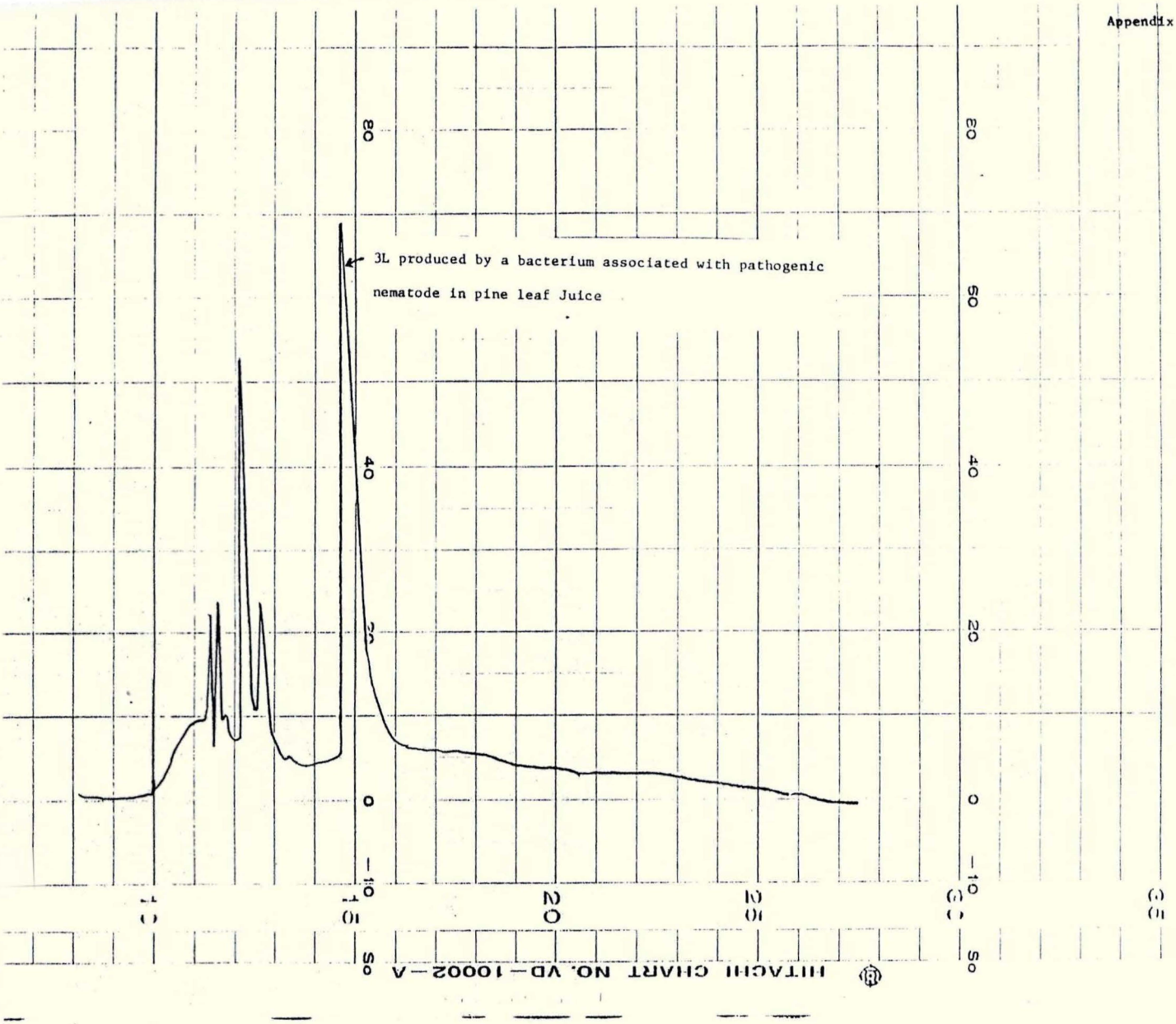
1981 (Kyushu F.T.B.I.)

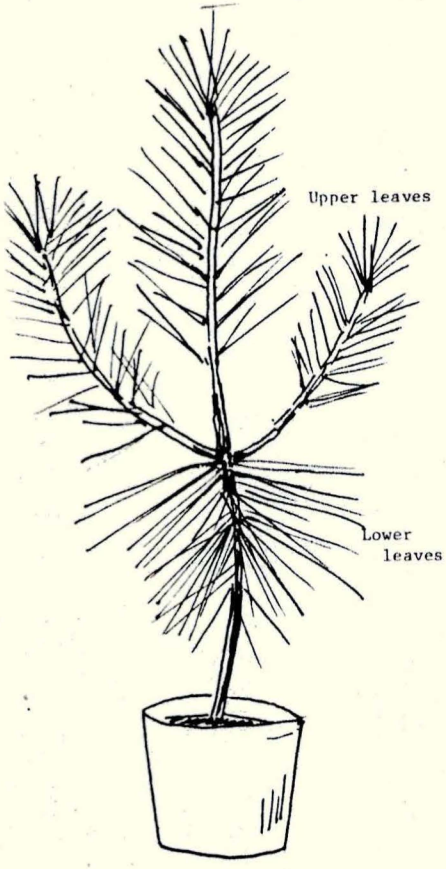
P.densiflora	273	10	3.7
P.thunbergii	936	1	0.1
Total	1209	11	0.9

PROCEDURE OF THE NEMATODES CULTIVATION




Benzoic acid
3L ?
Leaf extract from inoculated seedling
Leaf extract from healthy seedling


3L produced by a bacterium associated with pathogenic
nematode in pine leaf Juice
80
40
0
-10
60
50
40
20
0
-10
HITACHI CHART NO. YD-10002-A



Accumulation of benzoic acid in pine leaves of 2-year-old seedlings after inoculation with *Bursaphelenchus lignicolus*

Days after inoculation	Benzoic acid content (ug/g dry wt.)		Water content (%)	
	upper leaves	lower leaves	upper leaves	lower leaves
0	ND	ND	66.5	72.9
7	ND	ND	-	-
14	14.1	24.4	58.1	53.6
21	23.6	62.2	15.1	36.7
28	79.5	260.0	14.4	14.8
35	148.3	855.5	10.7	11.4

Pine seedlings were inoculated with ca.4000 of nematodes and placed in a green house. (Temperature: 28°C - 36°C)

Forest Resources of Iwata Prefecture, 1981

Forest statistic	National Forest	Private Forest	Total
Forest Area, ha	404,000	794,000	1,199,000
Volume, m^3			
P. densiflora	2,819,000	10,230,000	13,049,000
Larix sp.	2,434,000	6,250,000	8,684,000
Cryptomeria	2,050,000	13,532,000	15,582,000
Broadleaf tree	--	37,722,000	37,722,000
Area of reforestation, ha, 1975			
Cryptomeria			3,642
P. densiflora			3,392
Larix			663
Other			238
Area of reforestation, ha, 1979			
Cryptomeria			4,246
P. densiflora			5,505
Larix			325
Other			409

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